UNIVERSITY OF IOANNINA
SCHOOL OF HEALTH SCIENCES
DEPARTMENT OF BIOLOGICAL APPLICATIONS AND TECHNOLOGIES

STUDY ON THE BIOLOGY OF THE SYNGNATHIDAE FAMILY IN GREECE

LIOUSIA VARVARA

PHD THESIS

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European Union
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OPERATIONAL PROGRAMME
EDUCATION AND LIFELONG LEARNING
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MINISTRY OF EDUCATION & RELIGIOUS AFFAIRS
MANAGING AUTHORITY

NSRF 2007-2013
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ΠΑΝΕΠΙΣΤΗΜΙΟ ΙΩΑΝΝΙΝΩΝ
ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ
ΤΜΗΜΑ ΒΙΟΛΟΓΙΚΩΝ ΕΦΑΡΜΟΓΩΝ ΚΑΙ ΤΕΧΝΟΛΟΓΙΩΝ

ΜΕΛΕΤΗ ΤΗΣ ΒΙΟΛΟΓΙΑΣ ΤΩΝ ΕΙΔΩΝ ΤΗΣ ΟΙΚΟΓΕΝΕΙΑΣ ΤΩΝ SYNGNATHIDAE ΣΤΗΝ ΕΛΛΑΔΑ

ΛΙΟΥΣΙΑ ΒΑΡΒΑΡΑ

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

ΙΩΑΝΝΙΝΑ, 2015

European Union
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The figure in the front pages depicts a male individual of *Syngnathus abaster* species giving birth to his offspring. Copyright to Liousia Varvara

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Η παρούσα έρευνα έχει συγχρηματοδοτηθεί από την Ευρωπαϊκή Ένωση (Ευρωπαϊκό Κοινωνικό Ταμείο - ΕΚΤ) και από εθνικούς πόρους μέσω του Επιχειρησιακού Προγράμματος «Εκπαίδευση και Δια Βίου Μάθηση» του Εθνικού Στρατηγικού Πλαισίου Αναφοράς (ΕΣΠΑ) – Ερευνητικό Χρηματοδοτούμενο Έργο: Ηράκλειτος ΙΙ . Επένδυση στην κοινωνία της γνώσης μέσω του Ευρωπαϊκού Κοινωνικού Ταμείου.

«Η έγκριση της παρούσας Διδακτορικής Διατριβής από το Τμήμα Βιολογικών Εφαρμογών και Τεχνολογιών της σχολής Επιστημών Υγείας του Πανεπιστημίου Ιωαννίνων, δεν υποδηλώνει αποδοχή των γνωμών του συγγραφέως (Ν. 5343/1932, άρθρο 202, παραγρ. 2)».

Members of the examining committee

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Leonardos Ioannis, Professor, Department of Biological Applications and Technologies, University of Ioannina (Supervisor).

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ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ & ΤΕΧΝΟΛΟΓΙΩΝ
ΤΜΗΜΑ ΒΙΟΛΟΓΙΚΩΝ ΕΦΑΡΜΟΓΩΝ & ΤΕΧΝΟΛΟΓΙΩΝ

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Ιωάννινα 20-01-2009
Αριθμ.Πρωτ. 697

Προς τους κ.κ.:
1) Ιωάννη Λεονάρδο, Αναπληρωτή Καθηγητή του Τμήματος Βιολογικών Εφαρμογών & Τεχνολογιών του Παν/μίου Ιωαννίνων
2) Τριανταφυλλίδη Αλέξανδρο, Επίκουρο Καθηγητή του Τμήματος Βιολογίας Αριστοτελείου Πανεπιστημίου Θεσσαλονίκης
3) Κουτράκη Εμμανουήλ, Κύριο Ερευνητή του Ινστιτούτου Αλιευτικής Έρευνας και Τεχνολογίας του Εθνικού Ιδρύματος Αγροτικής Έρευνας

Σας πληροφορούμε ότι, μετά από εισήγηση του Επιβλέποντα κ. Ιωάννη Λεονάρδου και της Σ.Ε.Μ.Σ. του Τμήματος, η Προσωρινή Συνέλευση Ειδικής Σύνθεσης, στη Συνεδρίασή της αριθμ. 124/16-01-2009, σας όρισε μέλη τριμελούς Συμβουλευτικής Επιτροπής, για την εκπόνηση της Διδακτορικής Διατριβής της κ. **Βαρβάρας Λιούσια**.

Επίσης, ενέκρινε ως θέμα της διδακτορικής διατριβής το εξής: ***"Μελέτη της Βιολογίας των ειδών της οικογένειας των Sygnathidae στην Ελλάδα"***.

Κοινοποίηση:
- κ. Βαρβάρα Λιούσια

Με εντολή του Προέδρου
Η Γραμματέας του Τμήματος

ΑΝΝΑ ΥΦΑΝΤΗ

Τμήμα
Βιολογικών
Εφαρμογών
& Τεχνολογιών

ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ
ΠΑΝΕΠΙΣΤΗΜΙΟ ΙΩΑΝΝΙΝΩΝ
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Ιωάννινα, 19 Μαρτίου 2014
Αριθμ. Πρωτ.: 310

Προς τους κ.κ.
1. Ι. Λεονάρδο, Καθηγητή Τμήματος Βιολογικών Εφαρμογών & Τεχνολογιών, Π.Ι.
2. Ε. Κουτράκη, Ερευνητή, Ινστιτούτου Αλιευτικής Έρευνας, ΕΘΙΑΓΕ
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6. Κ. Σωτηρόπουλος Λέκτορας Τμήματος Βιολογικών Εφαρμογών & Τεχνολογιών, Π.Ι.
7. Γ. Κεχαγιά, Επικ. Καθηγητή Τμήματος Διαχείρισης Περιβάλλοντος και Φυσικών Πόρων, Παν/μιο Πατρών

ΘΕΜΑ: «Ορισμός Επταμελούς Εξεταστικής Επιτροπής για την κρίση της διδακτορικής διατριβής της κα. Βαρβάρας Λιούσια».

Σας γνωρίζουμε ότι η Γενική Συνέλευση Ειδικής Σύνθεσης του Τμήματος Βιολογικών Εφαρμογών και Τεχνολογιών στη Συνεδρίασή της αριθμ. 212/14-03-2014, σας όρισε μέλη της 7μελούς εξεταστικής επιτροπής για την κρίση της διδακτορικής διατριβής που εκπόνησε η κα. Βαρβάρα Λιούσια.

Πρόεδρος της Επιτροπής ορίζεται ο Καθηγητής του Τμήματος κ. Ι. Λεονάρδος.

Κοινοποίηση:
- κα. Βαρβράρα Λιούσια

Με εντολή του Προέδρου
Η Γραμματέας του Τμήματος

ΑΝΝΑ ΥΦΑΝΤΗ

ΠΡΑΚΤΙΚΟ

ΔΗΜΟΣΙΑΣ ΠΑΡΟΥΣΙΑΣΗΣ, ΕΞΕΤΑΣΗΣ ΚΑΙ ΑΞΙΟΛΟΓΗΣΗΣ ΔΙΔΑΚΤΟΡΙΚΗΣ ΔΙΑΤΡΙΒΗΣ

Σήμερα 30/4/2014 και ώρα 13:00 στην αίθουσα τηλεδιασκέψεων του κτηρίου του Τμήματος Φυσικής του Πανεπιστημίου Ιωαννίνων, πραγματοποιείται σύμφωνα με τα άρθρα 12 & 13 του Ν.2083/92, η διαδικασία της δημόσιας παρουσίασης, εξέτασης και αξιολόγησης της διδακτορικής διατριβής της υποψήφιας διδάκτορος του Τμήματος Βιολογικών Εφαρμογών και Τεχνολογιών **κ. Βαρβάρας Λιούσια**.

Την επταμελή εξεταστική επιτροπή, που συγκροτήθηκε με απόφαση της Γενικής Συνέλευσης Ειδικής Σύνθεσης (212/14-03-2014) του Τμήματος Βιολογικών Εφαρμογών & Τεχνολογιών, αποτελούν οι:

1. **Λεονάρδος Ιωάννης, Καθηγητής**, του Τμήματος Βιολογικών Εφαρμογών και Τεχνολογιών του Πανεπιστημίου Ιωαννίνων (Επιβλέπων).
2. **Κουτράκης Εμμανουήλ, Τακτικός Ερευνητής** του Ινστιτούτου Αλιευτικής Έρευνας Καβάλας, ΕΛΓΟ «ΔΗΜΗΤΡΑ» (Μέλος της τριμελούς συμβουλευτικής επιτροπής).
3. **Τριανταφυλλίδης Αλέξανδρος, Επίκουρος Καθηγητής** του Τμήματος Βιολογίας, του Αριστοτέλειου Πανεπιστημίου Θεσσαλονίκης (Μέλος της τριμελούς συμβουλευτικής επιτροπής).
4. **Αμπατζόπουλος Θεόδωρος, Καθηγητής** του Τμήματος Βιολογίας, του Αριστοτέλειου Πανεπιστημίου Θεσσαλονίκης (Μέλος της επταμελούς εξεταστικής επιτροπής).
5. **Τσίκληρας Αθανάσιος, Επίκουρος Καθηγητής** του Τμήματος Βιολογίας, του Αριστοτέλειου Πανεπιστημίου Θεσσαλονίκης (Μέλος της επταμελούς εξεταστικής επιτροπής).
6. **Κεχαγιάς Γεώργιος, Επίκουρος Καθηγητής** του Τμήματος Διαχείρισης Περιβάλλοντος και Φυσικών Πόρων του Πανεπιστημίου Πατρών, (Μέλος της επταμελούς εξεταστικής επιτροπής).
7. **Σωτηρόπουλος Κωνσταντίνος, Λέκτορας** του Τμήματος Βιολογικών Εφαρμογών και Τεχνολογιών του Πανεπιστήμιο Ιωαννίνων, (Μέλος της επταμελούς εξεταστικής επιτροπής).

Το θέμα της διατριβής που εκπόνησε και παρουσίασε σήμερα η κ. Βαρβάρα Λιούσια είναι:

«ΜΕΛΕΤΗ ΤΗΣ ΒΙΟΛΟΓΙΑΣ ΤΩΝ ΕΙΔΩΝ ΤΗΣ ΟΙΚΟΓΕΝΕΙΑΣ ΤΩΝ SYNGNATHIDAE ΣΤΗΝ ΕΛΛΑΔΑ»

Ο Πρόεδρος της εξεταστικής επιτροπής, ο κ. **Ι. Λεονάρδος**, καλεί την υποψήφια να αναπτύξει το θέμα της διδακτορικής διατριβής.

Ακολουθεί ανάπτυξη και παρουσίαση του θέματος από την υποψήφια.

Στη συνέχεια, αφού η υποψήφια απαντά σε σχετικές ερωτήσεις, η εξεταστική επιτροπή αποσύρεται και εισέρχεται στη διαδικασία αξιολόγησης της υποψήφιας και της τελικής κρίσης της διδακτορικής διατριβής.

Μετά από συζήτηση, η εξεταστική επιτροπή, κατέληξε στα ακόλουθα:

Ο πρώτος εξεταστής και πρόεδρος της εξεταστικής επιτροπής και επιβλέπων της διατριβής κ. **Ι. Λεονάρδος**

1. Διαπιστώνει ότι:

α) Η παρουσίαση και ανάπτυξη του θέματος της διδακτορικής της διατριβής ήταν αριστη

β) Η επιστημονική κατάρτιση της υποψήφιας είναι πολυ καλη

γ) Η συγγραφή της διατριβής έγινε με τρόπο που δείχνει αρτια και ενημέρωση της υποψήφιας πάνω στη βιβλιογραφία τη σχετική με το θέμα της διατριβής.

2. Κρίνει ότι:

α) Η διατριβή είναι προϊόν μακρόχρονης κοπιώδους προσπάθειας και καταλήγει σε σημαντικά αποτελέσματα τα οποία προάγουν την επιστήμη.

β) Η επάρκεια της υποψήφιας στο γνωστικό αντικείμενο της διατριβής είναι πολυ καλη

ο κ. Λεονάρδος δύναται να παρατηρήσει ναι

Εισηγείται βαθμό αριστα (.....)

Ο δεύτερος εξεταστής και μέλος της συμβουλευτικής επιτροπής κ. **Κουτράκης Εμμανουήλ**

1. Διαπιστώνει ότι:

α) Η παρουσίαση και ανάπτυξη του θέματος της διδακτορικής της διατριβής ήταν πολυ καλη

β) Η επιστημονική κατάρτιση της υποψήφιας είναι αρτια

γ) Η συγγραφή της διατριβής έγινε με τρόπο που δείχνει πολυ καλη και ενημέρωση της υποψήφιας πάνω στη βιβλιογραφία τη σχετική με το θέμα της διατριβής.

2. Κρίνει ότι:

α) Η διατριβή είναι προϊόν μακρόχρονης κοπιώδους προσπάθειας και καταλήγει σε σημαντικά αποτελέσματα τα οποία προάγουν την επιστήμη.

β) Η επάρκεια της υποψήφιας στο γνωστικό αντικείμενο της διατριβής είναι πολυ καλη

ο κ. Κουτράκης δύναται να παρατηρήσει ναι

Εισηγείται βαθμό Αριστα (10)

Ο τρίτος εξεταστής και μέλος της συμβουλευτικής επιτροπής κ. **Τριανταφυλλίδης Αλέξανδρος**

1. Διαπιστώνει ότι:

α) Η παρουσίαση και ανάπτυξη του θέματος της διδακτορικής της διατριβής ήταν ...άριστη

β) Η επιστημονική κατάρτιση της υποψήφιας είναι ...πολύ καλή

γ) Η συγγραφή της διατριβής έγινε με τρόπο που δείχνει άρτια γνώση και ενημέρωση της υποψήφιας πάνω στη βιβλιογραφία τη σχετική με το θέμα της διατριβής.

2. Κρίνει ότι:

α) Η διατριβή είναι προϊόν μακρόχρονης κοπιώδους προσπάθειας και καταλήγει σε σημαντικά αποτελέσματα τα οποία προάγουν την επιστήμη.

β) Η επάρκεια της υποψήφιας στο γνωστικό αντικείμενο της διατριβής είναι ...άριστη

Ο κ. Τριανταφυλλίδης ...δεν έχει να παρατηρήσει ...κάτι

Εισηγείται βαθμό άριστη (10).

Ο τέταρτος εξεταστής και μέλος της εξεταστικής επιτροπής κ. **Αμπατζόπουλος Θεόδωρος**

1. Διαπιστώνει ότι:

α) Η παρουσίαση και ανάπτυξη του θέματος της διδακτορικής της διατριβής ήταν .ικανοποιητική

β) Η επιστημονική κατάρτιση της υποψήφιας είναι ..καλή

γ) Η συγγραφή της διατριβής έγινε με τρόπο που δείχνει επαρκή και ενημέρωση της υποψήφιας πάνω στη βιβλιογραφία τη σχετική με το θέμα της διατριβής.

2. Κρίνει ότι:

α) Η διατριβή είναι προϊόν μακρόχρονης κοπιώδους προσπάθειας και καταλήγει σε σημαντικά αποτελέσματα τα οποία προάγουν την επιστήμη.

β) Η επάρκεια της υποψήφιας στο γνωστικό αντικείμενο της διατριβής είναι ...μέτρια.

ο. κ. Αμπατζόπουλος .....έχει να παρατηρήσει...ότι η υποψήφια δεν έδωσε ικανοποιητικές απαντήσεις σε πολλές από τις ερωτήσεις που της είχαν κάνει τα μέλη της εξεταστικής επιτροπής. Επίσης, το κείμενο πρέπει να διορθωθεί γιατί χρησιμοποιούνται με λανθασμένο τρόπο διάφοροι όροι.

Εισηγείται βαθμό λίαν καλώς (8,4).

Ο πέμπτος εξεταστής και μέλος της εξεταστικής επιτροπής κ. **Τσίκληρας Αθανάσιος**

1. Διαπιστώνει ότι:

α) Η παρουσίαση και ανάπτυξη του θέματος της διδακτορικής της διατριβής ήταν ...πολύ καλή

β) Η επιστημονική κατάρτιση της υποψήφιας είναι ...καλή αρκετά καλή γνώση

γ) Η συγγραφή της διατριβής έγινε με τρόπο που δείχνει ..... και ενημέρωση της υποψήφιας πάνω στη βιβλιογραφία τη σχετική με το θέμα της διατριβής.

2. Κρίνει ότι:

α) Η διατριβή είναι προϊόν μακρόχρονης κοπιώδους προσπάθειας και καταλήγει σε σημαντικά αποτελέσματα τα οποία προάγουν την επιστήμη.

β) Η επάρκεια της υποψήφιας στο γνωστικό αντικείμενο της διατριβής είναι ..μέτρια

ο κ. Τσίκληρας ....έχει να παρατηρήσει ..όλη οι απαντήσεις της υποψήφιας στις ερωτήσεις της επιτροπής ήταν ανεπαρκείς.

ΛΙΑΝ ΚΑΛΟΣ

Εισηγείται βαθμό ..... (8.4).

Ο έκτος εξεταστής και μέλος της εξεταστικής επιτροπής κ. **Κεχαγιάς Γεώργιος**

1. Διαπιστώνει ότι:

α) Η παρουσίαση και ανάπτυξη του θέματος της διδακτορικής της διατριβής ήταν λάθη πολύ καλή

β) Η επιστημονική κατάρτιση της υποψήφιας είναι ..άρτια άριστη γνώση

γ) Η συγγραφή της διατριβής έγινε με τρόπο που δείχνει..... και ενημέρωση της υποψήφιας πάνω στη βιβλιογραφία τη σχετική με το θέμα της διατριβής.

2. Κρίνει ότι:

α) Η διατριβή είναι προϊόν μακρόχρονης κοπιώδους προσπάθειας και καταλήγει σε σημαντικά αποτελέσματα τα οποία προάγουν την επιστήμη.

β) Η επάρκεια της υποψήφιας στο γνωστικό αντικείμενο της διατριβής είναιπολύ καλή

ο κ. Κεχαγιάς δε..έχει να παρατηρήσει κάτι

Εισηγείται βαθμό Άριστα..... (10).

Ο έβδομος εξεταστής και μέλος της εξεταστικής κ. **Σωτηρόπουλος Κωσταντίνος**

Ο δεύτερος εξεταστής

1. Διαπιστώνει ότι:

α) Η παρουσίαση και ανάπτυξη του θέματος της διδακτορικής της διατριβής ήταν ……άριστη

β) Η επιστημονική κατάρτιση της υποψήφιας είναι .καλή

γ) Η συγγραφή της διατριβής έγινε με τρόπο που δείχνει καλή γνώση ………και ενημέρωση της υποψήφιας πάνω στη βιβλιογραφία τη σχετική με το θέμα της διατριβής.

2. Κρίνει ότι:

α) Η διατριβή είναι προϊόν μακρόχρονης κοπιώδους προσπάθειας και καταλήγει σε σημαντικά αποτελέσματα τα οποία προάγουν την επιστήμη.

β) Η επάρκεια της υποψήφιας στο γνωστικό αντικείμενο της διατριβής είναι αρκετά καλή

ο κ. Σωτηρόπουλος ….. έχει να πρατηρήσει…το σημαντικό όγκο εργασίας και την αφοσίωση στο θέμα και τη διεκπεραίωσή του.

Εισηγείται βαθμό Άριστα 9.0 ………….. (….).

Με βάση τα ανωτέρω τα μέλη της εξεταστικής επιτροπής αποδέχτηκαν τη διδακτορική διατριβή της **κ. Βαρβάρας Λιούσια** και εισηγούνται την απονομή του τίτλου του διδάκτορα με βαθμό ....Άριστα

Τα μέλη της Εξεταστικής Επιτροπής

Ιωάννης Λεονάρδος
Καθηγητής
(Επιβλέπων)

1) Λεονάρδος Ιωάννης Καθηγητής

2) Κουτράκης Εμμανουήλ, Τακτικός Ερευνητής

3) Τριανταφυλλίδης Αλέξανδρος Επίκ. Καθηγητής

4) Αμπατζόπουλος Θεόδωρος. Καθηγητής

5) Τσίκληρας Αθανάσιος Επικ. Καθηγητής

6) Κεχαγιάς Γεώργιος Επικ. Καθηγητής

7) Σωτηρόπουλος Κωσταντίνος, Λέκτορας

Published papers and conference communications with data from the doctoral thesis: Δημοσιευμένες εργασίες και εργασίες σε συνέδρια που προέκυψαν με δεδομένα από τη διδακτορική διατριβή:

Liousia V., Leonardos I.D., Koutrakis E. Triantafyllidis A. (2015). Genetic structure of two congeneric and sympatrically occurring pipefish (*Syngnathus abaster* and *Syngnathus typhle*) along the Greek coastline; a story of two tails. (submitted).

Liousia V., Batziakas S., Panagiotou N., Daouti I., Koutrakis E. and Leonardos I.D. (2012). Length-weight relations of 22 fish species from the littoral zone of the Eastern Ionian Sea, Greece. Acta Ichthyologica et Piscatoria 42 (1), 69-72

Liousia V., Triantafyllidis A. and Leonardos I.D. Biometric and genetic analyses of Syngnathus species in Greece. 2nd Syngnathid Biology International Symposium, Faro, Portugal 25- 29 March 2013.

Liousia V. and Leonardos I.D. "Biology of *Syngnathus abaster* in Eastern Ionian Sea, Greece" Proceedings of the12th International Congress on the Zoogeography and Ecology of Greece and Adjacent Regions, Athens, Greece, June 18-22, 2012 Athens.

Liousia V., Anastasiadou C. and Leonardos I.D. "Contribution in the Crustacean Decapoda of the E. Ionian Sea" Proceedings of the Crustacean Society Summer Meeting and the 10th Colloquium Crustacea Decapoda Mediterranean, Athens, Greece, June 3-7, 2012.

Kokoli F., **Liousia V.** and Leonardos I.D. "Population structure and biological features of *Atherina boyeri* (Risso, 1939) in the littoral zone of the N.Ionian Sea" 33rd Scientific Conference of the Hellenic Society for Biological Sciences, Edessa May 19-21, 2011.

Liousia V., Triantafyllidis A. and Leonardos I.D. "Biology of *Syngnathus typhle* in Greece" The 1st Syngnathid Biology International Symposium, Fiskebascskil, Sweden, April 25-29, 2011.

to Kostas and Maro

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Preface

They say that it's the journey that counts and the last 5 years have been an amazing one! With its ups and downs, with moments of happiness and frustration, with excitements and disappointments and so many experiences, emotions and memories that fill my head and soul. Through this journey, I matured not only scientifically, but as a person too, by reaching out my limits, learning how to move on no matter how hard it was, appreciating each small victory, asking for help and giving it back, building new relationships and working with so many different people. All of this would not have been possible without the help of all those people who supported me all the way or in crucial and difficult moments. The next pages are devoted to all of you as I want to say thank you from the bottom of my heart for all these things you have done for me.

I could not help but starting with my supervisor, Professor **Ioannis D. Leonardos.** You were my professor as an undergraduate student. I remember being amazed in your lectures by your eloquence, passion and knowledge on zoology. You took me under your wings for the undergraduate diploma thesis, trusted me with a master thesis, worked together on many research programs and now here we are, finishing the PhD thesis. I don't think that words are strong enough to express the levels of gratitude that I feel. THANK YOU FROM THE BOTTOMS OF MY HEART. You are my MENTOR, the father of my lab family, the person who trusted me all these years and challenged me the most. I hope that someday I will be able to live up to your expectations. THANK YOU….

I was lucky enough and my supervising committee, the Assistant Professor Alexandros Triantafyllidis and the Senior Researcher Emmanouil Koutrakis had an acting part in my PhD. Despite being in different institutes and cities their guidance played a pivotal role in the process of my research.

Dr. Triantafyllidis, for the last two years you stood by me as an actual supervisor, guiding me through the DNA world, tolerating with my stubbornness (quoting your exact words) and helping me understand and overcome my deficiencies. THANK YOU for believing in me when I did not. THANK YOU for being always there for me.

Dr. Koutrakis, you were always so kind to me! During that summer that I was searching all over Greece for pipefish you helped to pull through the E-Mediterranean-Thrace region. Also you were patient enough to teach me how to sort juvenile mugilids even if that meant staying till late in the lab away from your family. THANK YOU! Your opinion is always valuable!

One way or another, my examination committee had been involved in my PhD. **Dr. Abatzopoulos**, you were kind enough to let me work on LICOR Bioanalyser during the experiments of the microsatellite analysis. Your door was always open for me and I would like to thank you for that.

Dr. Tsikliras, you were always so kind with me. I appreciate our talks on many biological aspects. You helped me put things into perspective and understand what people will think of my work. Thank you for that.

Dr. Sotiropoulos, even though you are new professor in our department your door was always open for me. I appreciate our talks and the fact that you were always willing to help me. Thank you very much.

Dr. Kehagias thank you very much for honoring me and becoming a part of my examination committee.

At this point I would like to thank my second family; my **lab family.** All the past and present members that have guided and accompanied me all those years. Dr. Liasko, thank you for teaching me $P<0.05$! Dr. Anastasiadou you are the mother of our lab. Doti, Olga and Evi, not only co-workers but also dear friends. Olga, you are the aunt of my pipefish! Chris you were my travel body during that long summer. Miranda, you were always there for me, always knowing the right person…. Alex you always made me smile. Argyris, you are my lab brother! Dr. Kagalou, thank you for being always so kind. Anthi better late than never… Florianna, Konstantina, Strato, Niko†, Eirini, Andrea, Louka and Gavril thank you for your excellent collaboration and all the field trips.

Besides my lab I was lucky enough to work in **Dr. Triantafyllidis lab** for almost half a year. I would like to thank from the bottom of my heart **Dr. N. Karaiskou** for teaching me how to work in the panic room! You were always patient with me and always a friendly face to turn to. Kostas, Stella, Antonis, Mariallena, thank you for making my staying there easy and for taking my mind off the panic room. Natasha thank you for the excellent collaboration.

I would also like to thank **Assistant. Prof. E. Chatziloukas and A.S. Afendra** for letting me use their lab equipment. All the **members of their lab** made my life easier just by waiting with me by the electrophoresis and always taking my mind away and making me smile. Tasos, Panos, Vagelis, Tina, Maria and Michalis thank you very much.

Besides my coworkers I would like to thank all my **friends** for putting up with me all those years. I want to apologize for missing your birthdays and name days, never being in time, always worrying for "some fish" but never for you... Thank you for not turning your back on me. Thank you for being my friends!

And of course I would like to thank my **parents** for supporting me all those years. But most importantly, I would like to apologize for being such a lousy daughter and always absent from the "little things". I hope I will make up to you…

Last but not least I would like to thank my husband and my sister. **Kostas** and **Maria** you were always there for me, through good and bad times. You never let my hand and I will never forget that. THANK YOU!!!!!!!!!!

Abstract

Seahorses, pipefishes, seadragons and pipehorses are members of the family of Syngnathidae. They are resident species, inhabiting vegetated coastal ecosystems and have a word wide distribution. The most interesting aspects of their biology are a) male pregnancy and parental care, b) sex role reversal and c) complex mating system. Despite the fact that studies on the biology, mating system and phylogenetic relationships of European syngnathids have significantly increased over the last decades, they mostly focus on populations at different/ major biogeographical zones and therefore provide data only on large scale differences.

Due to the high degree of vegetation along the Greek coastline, nine syngnathid species occur sympatrically: *Hippocampus hippocampus*, *Hippocampus guttulatus*, *Nerophis ophidion*, *Syngnathus abaster*, *Syngnathus acus*, *Syngnathus phlegon*, *Syngnathus typhle, Syngnathus taenionutus*, and *Syngnathus tenuirostris*. Studies on the biology of these species in the Ionian and the Aegean Sea are rare and in many cases any available information is the result of bycatch. Also, despite the fact that existing phylogenetic studies at a European level tried to cover the species full distributional range, sampling regions are lacking in the Eastern Mediterranean part.

The lack of available information on the biology and phylogeny of syngnathids along the Greek coastline is a major gap in the overview of the species biology and evolution as the Ionian and Aegean Seas: a) consist of a wide range of ecosystems regarding biotic, abiotic, physicochemical and other environmental factors, b) constitute two major biogeographical zones c) are important ecosystems of the E. Mediterranean Sea as they are in close proximity to the Marmara, Black and Adriatic Seas

Taking these factors into consideration, the aim of the present study was to examine the biology, the genetic and phenotypic structure, and the mating system of the sympatrically occurring syngnathids along the Greek coastline. The two most abundant species of the Greek coastline – *Syngnathus abaster* and *Syngnathus typhle*- were used as model organisms.

The biology of the two species was assessed by the examination of the relative abundance, population structure, sex ratio, operational sex ratio, length at maturity, length-weight relationships, and gonadosomatic and hepatosomatic index in two ecosystems of the N. Ionian Sea (Drepano and Neochori). These habitats vary in abiotic factors (type and coverage of habitat, exposure to the open sea etc.) and are almost 80 km apart.

A total of 578 specimens of *S. abaster* species were caught in Drepano (n=313) and Neochori (n=265) stations during the present study. The relative abundance of sampled specimens was not statistically different between the two stations. At the same time, the number of caught individuals at each period differed in both stations indicating a seasonal migration pattern, i.e. overwintering in deeper waters. In Drepano station the population

composed of specimens from 28.0 mm (juvenile) to 253.0 mm (female). During the same period, in Neochori station the shortest total length (23.0 mm) was recorded in a juvenile individual while the longest (238.0 mm) in a male. The length frequency distribution of adult and unsexed specimens in Drepano and Neochori stations revealed two length classes indicating the occurrence of two cohorts per year. The first cohort constituted mainly of unsexed and adult individuals smaller than 120 mm (0+), while the second cohort consisted only of adult individuals larger than 120 mm (1+). Therefore, the species lifespan most probably exceeded 12 months and reached 18-20 months, similarly to the Adriatic Sea population. In both stations males and females exhibited positive allometric growth pattern, whereas unsexed specimens presented an isometric pattern. The overall sex ratio of *S. abaster* males to females and the operational sex ratio (ratio of non-brooding to active female individuals across the breeding period) were female biased in both stations. This outcome was expected as female biased operational sex ratio is an indicator of sex role reversal. *S. abaster* individuals matured and were ready to reproduce when they reached approximately 70 mm. Reproductive period started in early spring (March or April) and lasted until early October as indicated by the formation of the brooding pouch, the presence of pregnant males and the values of the gonadosomatic index. Furthermore, the values of gonadosomatic index implied synchronization of male pregnancy in Neochori station and unsychronization in Drepano.

A total of 724 specimens of *S. typhle* species were caught in Drepano (N=287) and Neochori (N=437) stations during the present study. The relative abundance of sampled specimens was not statistically different between the two stations. However, the abundance of *S. typhle* in both stations varied during the present study. A seasonal migration pattern was most possibly the cause for this variation. The population in Drepano station composed of specimens from 33.0 mm (unsexed) to 243.0 mm (female). At the same time, in Neochori station the shortest individual was unsexed (25.0 mm), while the longest female (188.0 mm). The length frequency distribution of *S. typhle* in both stations indicated that the species life span consisted of two and possibly up to four age classes. The first cohort consisted mainly of unsexed and a few adult individuals with total length shorter than 80 mm (0+). The second, third and fourth cohorts consisted of adult individuals with total length shorter than 120 mm (1+), 160 mm (2+), and larger than 190 mm (> 2+) respectively. Among adult individuals, both males and females displayed a positive allometric growth pattern in both stations. *S. typhle* individuals in N. Ionian Sea matured when they reached about 75 mm. The operational sex ratio in both stations was female biased. This outcome was expected as the species is sex- role reversed and female biased operational sex ratio is an important indicator of this behavior. Reproductive period started in early spring and lasted until October. The duration of the reproductive period was indicated by the formation of the brooding pouch, the presence of pregnant males and the values of the gonadosomatic index. The values of the GSI further indicated that individuals in Drepano station breed in continuous batches throughout the reproductive period while in Neochori station two and possibly three major brooding batches occurred.

The genetic structure of the two species was examined using the mitochondrial DNA control region and the nuclear locus A1. Samples were collected along the Greek coastline from June to August 2010. Localities depicted the range of the species distribution along the major biogeographical basins in the mainland coastline of Greece. Both mtDNA and nDNA analysis verified the lack of haplotype sharing between the two species. Thus the phenomenon of hybridization was excluded. Both species were found to constitute monophyletic clades.

Along the Greek coastline, both mtDNA and nDNA analyses revealed in most cases a striking lack of haplotype sharing between Ionian and Aegean Sea individuals of *S. abaster* species. This fact indicated a significant distinction in the species genetic structure between the two Seas. A possible break could be located in Southern Peloponnese Peninsula. Against the general pattern, phylogenetic tree and network analysis revealed the presence of few common haplotypes between the Ionian (Kalogria samples) and the N.C. Aegean Sea.

Taking all these factors into consideration, the genetic structure of *S. abaster* along the Greek coastline was more probably justified by the presence of at least two refugia (one for the Aegean and a second one for the Ionian Seas populations) during the glacial-interglacial activities. The ancestral populations of these refugia recolonized the two Seas. Even though geographical barriers, hydrological features and reduced dispersal ability - due to the bony armor along syngnathids body and the benthic behavior encountered in both juvenile and adult specimens - promoted the isolation of the Ionian and Aegean Sea populations, there was some (reduced) gene flow. This admixture could have occurred either during the postglacial recolonization process or at a latter point due to passive transportation. However, this reduced gene flow was not sufficient enough to induce homogenization and ultimately led to the distinct clades of the Ionian and Aegean Sea populations.

Along the Greek coastline the genetic structure of *S. typhle* was incongruent between mtDNA and nDNA markers. Only mtDNA analysis revealed a pattern of genetic structuring uncorrelated though to geographical barriers. Despite the existence of genetic structure, shared haplotypes were recorded even between distant populations. These shared haplotypes could be the result of a common gene pool (common refugium during the Pleistocene period) and/or indicate passive transportation of juveniles in the floating vegetation. nDNA analysis revealed high levels of connectivity and panmixia. The incongruent pattern of genetic variation and structuring revealed by the two markers could be attributed to their different evolution rates and/or to a larger dispersal ability of females compared to males, or it could be an artifact due to the low number of samples sampling.

At a European level mtDNA analysis indicated that the Greek haplotypes forming the E. Mediterranean clade, were separated from the W. Mediterranean and Adriatic Seas haplotypes and showed a limited affinity to the Marmara-Black Seas. In agreement with results at Greek scale, nDNA marker analysis revealed a very shallow genetic structuring

suggesting that across the species range the southern populations (including Greek samples) are less isolated and related than the northern. These patterns, both at a regional and broader scale, seem to support the hypothesis of a common refugium in the E. Mediterranean for both Ionian and Aegean Sea population during the Pleistocene period.

Morphological differences were investigated with the use of a fifteen landmark based morphometric protocol and seven meristic characters. Canonical Variate Analysis, revealed that *S. typhle* had a more elongated snout and main body, whereas its trunk was shorter compared to *S. abaster*. The difference of the main body and the trunk between the two species was also supported by meristic characters. *S. typhle* had more rings in the main body (predorsal) and less in the trunk (underdorsal and postdorsal) compared to *S. abaster*. The observed snout shape changes could probably be attributed to variations in the foraging ecology of the two species. In particular, the long snout of *S. typhle* could account for the species preference on fast moving and large sized pelagic preys. On the contrary the short snout of *S. abaster* is most probably associated with a preference on little prey hidden in the vegetation. Morphological comparison of *S. abaster* and *S. typhle* species indicated that the two species are completely separated, without any intermediate morphotypes.

Sexual dimorphism analysis indicated that females of *S. abaster* and *S. typhle* had a more elongated and thicker snout and main body and at the same time a more slender trunk compared to males. This outcome was associated to the fact that males needed more space to carry their embryos (thicker trunk) while females had larger main body in order to produce and store as many eggs as possible. The difference in the snout could be related to different feeding habits between the two sexes.

The phenotypic variation among individuals of *S. abaster* and *S. typhle* showed that both species form two morphometricaly distinct populations: i) Aegean and ii) Ionian Sea. For *S. typhle*, the species distinct morphological clades contradict the significant yet unrelated to geographic distances genetic structure found based on mtDNA analysis .The analysis of *S. abaster* showed that the individuals in the Ionian Sea had a more slender body and elongated snout compared to the Aegean Sea's. Additionally, apart from major geographical differentiation, even more groups were discovered: The population of Ionian Sea was further divided into three distinct groups: i) N. Ionian Sea- Katakolo, ii) Kotichi-Kalogria group and iii) Neochori (Amvrakikos Gulf). The shape changes associated with the CV axes showed that: i) individuals from Kotichi- Kalogria and Neochori group had a more elongated body and a thicker trunk than N. Ionian Sea- Katakolo group, ii) individuals from Kotichi- Kalogria group had a larger head, a more elongated snout and main body compared to the individuals from the rest of the groups. The population of the Aegean Sea was further divided into three distinct groups: i) N.W. and C. Aegean Sea, ii) N.E. Aegean Sea and iii) S. Aegean Sea. The shape changes associated with the CV axes showed that: i) individuals from the N.E. Aegean group had larger head, smaller main body and slender trunk compared to the N.W. and C. Aegean Sea ii) individuals from the S. Aegean Sea group had a more elongated snout and main body compared to the N. Aegean Sea.

The species morphometric pattern (Ionian-Aegean sea distinct populations) was in accordance with the results of the molecular analysis. However, the clades formed within the Ionian and Aegean Sea were not identical with the species genetic structure. Since morphometric characters are only partially genetically determined, the above noticed morphological pattern could be a form of local adaptation to the variable environmental and physicochemical conditions of Ionian and Aegean Sea. In particular, the groupings within the Ionian and Aegean Seas corresponded to different types of ecosystems.

The process of male pregnancy and the genetic mating system of *S. abaster* and *S. typhle* were examined in individuals collected in Drepano station (N. Ionian Sea) in the first half of the reproductive period of 2012. Pregnant males of both species were brought to the laboratory and were kept in aquariums until they released all the embryos of their brood pouch.

Eleven pregnant males of *S. abaster* and three of *S. typhle* gave birth under laboratory conditions in the present study. During pregnancy the brood pouch of the two species changed both in color and texture. From a stiff structure with similar color to the rest of the body in the beginning of the pregnancy, it became dark colored and soft before parturition. On the day of the parturition males displayed reduced mobility and their pouches were almost ready to open. Most of the pregnant males of both species released their embryos in the night or early in the morning in consecutive sporadic batches by sharp bending movements of their body. After approximately 24 hours fully formed juveniles, resembling the adults, were released from the marsupium. Newborn juveniles of *S. abaster* (average number of 43 juveniles per male) spent most of the time near the bottom hiding or swimming in the sand, with only some sporadic movements towards the surface; benthic behavior. Contrary, newborn juveniles of *S. typhle* (average number of 43 juveniles per male) exhibited a vertical swim-up behavior near the surface of the aquarium; benthopelagic behavior. The total length, total weight and number of dorsal fin rays varied between the juveniles of both species. Also, their coloration varied from a light shade (light green or brown), to a darker (dark green or brown) or even black. However, in general, no important statistical differences were recorded in the total length, total weight and number of dorsal fin rays of different colored juveniles.

Four microsatellite loci -S. abas3, S.abas4, S.abas7 and S.abas9- were used to perform parentage analysis and assess the genetic mating system of *S. abaster* and *S. typhle* species. Eight selected pregnant males of *S. abaster*, a subset of their offspring (N=249) and thirty four wild individuals from Drepano station were successfully genotyped at each of the four loci. Colony software assigned all embryos to their known father without any mismatches. In total, according to the reconstruction of maternal genotypes at least twenty eight females contributed to the broods of the pregnant males. At the same time, each male received eggs from more than one female ranging from three to six mates. Within each brood, the number of offspring sired by each mother varied from one to twenty four. Among the sired juveniles statistically important differences were recorded in their total length and the number of dorsal fin rays.

Three pregnant males of *S. typhle*, a subset of their offspring (N=78) and eighteen individuals of the wild population (WP) were successfully genotyped at each of the four loci. Colony software assigned all embryos to their known father without any mismatches. At least seven females contributed to the broods of pregnant males. Two of the three males received eggs from multiple females (up to three) while one male received eggs only from one female. Within each birth, the number of offspring sired by different mothers varied from one to twenty nine. No sign of females multiple mating was noted since no offspring of different males with identical mother genotypes was found.

A subset of 1st day juveniles of *S. abaster* (n=278) and *S. typhle* (n=92) species were examined morphometricaly. The landmarks that were selected were the same as the ones used in adults' morphometric analysis. Linear Discriminant Analysis (LDA) showed a considerable difference among the 1st day juveniles of the two species, defining two non-overlapping groups: i) *S. typhle* species and ii) *S. abaster* species. The shape changes associated with the linear function axis showed that *S. typhle* specimens had more elongated snout, elongated and thinner main body, whereas their trunk was shorter compared to *S. abaster*. These differences are also, distinct traits between adult specimens of the two species. The fact that major interspecies differences were detectable from the first day juveniles indicated that *S. abaster* and *S. typhle* newborns are fully formed individuals, closely resembling adult fish.

In conclusion, the comparative study of the two congeneric and sympatrically occurring syngnathid species revealed some remarkable similarities and striking differences. In regard to the biology of *S. abaster* and *S. typhle*, both species seem to have established breeding non-competing populations in Drepano and Neochori ecosystems. The lack of difference in the population structure, growth pattern and reproduction period of both species in the examined stations indicated absence of local adaptations. The genetic mating system of *S. abaster* confirmed the species polygynandrous behavior with a higher number of females donors revealed so far. At the same time *S. typhle* was characterized as polygynous. Phylogenetic and morphometric relationship studies showed that *S. abaster* and *S. typhle* species, even though congeneric and sympatric along the coastline of Greece, are genetically and morphologically distinct. No intermediate haplotypes or morphotypes were found between the two species, indicating lack of contemporary hybridization. However a different pattern of intraspecies differentiation was observed revealing the effect of past and more contemporary drives.

Περίληψη

Η οικογένεια των Συγναθιδών αποτελείται από τους ιππόκαμπους, τις σακοράφες, τους θαλάσσιους δράκους και τους ταινιόμορφους ιππόκαμπους. Τα μέλη της παρουσιάζουν ευρεία γεωγραφική κατανομή και απαντώνται κυρίως σε παράκτια οικοσυστήματα με πλούσια βλάστηση. Ιδιαίτερο ενδιαφέρον στη βιολογία τους παρουσιάζουν η αντρική κύηση και η γονική φροντίδα, οι αντεστραμμένοι φυλετικοί ρόλοι και η πολύπλοκη αναπαραγωγική συμπεριφορά. Τα χαρακτηριστικά αυτά έχουν προκαλέσει το έντονο ενδιαφέρον της επιστημονικής κοινότητας τις τελευταίες δεκαετίας. Παρ όλα αυτά οι μελέτες που πραγματοποιούνται αφορούν κυρίως μεμονωμένους πληθυσμούς διαφορετικών βιογεωγραφικών περιοχών. Συνεπώς υπάρχει ένα σημαντικό κενό στη κατανόηση της βιολογίας και της πληθυσμιακής-γενετικής σύστασης των ειδών αυτών εντός της ίδιας ή γειτονικών βιογεωγραφικών ζωνών.

Στον ελλαδικό χώρο, απαντώνται εννιά είδη της οικογένειας των Συγναθιδών: *Hippocampus hippocampus*, *Hippocampus guttulatus*, *Nerophis ophidion*, *Syngnathus abaster*, *Syngnathus acus*, *Syngnathus phlegon*, *Syngnathus typhle*, *Syngnathus taenionutus* and *Syngnathus tenuirostris*. Παρά την αφθονία των ειδών στο Ιόνιο και το Αιγαίο Πέλαγος οι διαθέσιμες πληροφορίες σχετικά με τη βιολογία τους, τη κατάσταση των πληθυσμών τους, τις φυλογενετικές τους σχέσεις κ.α. είναι σπάνιες και συνήθως εκμαιεύονται δευτερογενώς από μελέτες σχετικές με άλλα είδη ιχθύων. Σε ευρωπαϊκό επίπεδο, παρά το γεγονός ότι οι υφιστάμενες φυλογενετικές μελέτες, προσπαθούν να καλύψουν όσο το δυνατόν μεγαλύτερο τμήμα της κατανομής των ειδών, δεν υπάρχουν επαρκή δεδομένα για την Α. Μεσόγειο. Συγκεκριμένα, μέχρι στιγμής έχουν μελετηθεί πληθυσμοί των συγκεκριμένων ειδών: i) μόνο από μία περιοχή του Αιγαίου Πελάγους ή ii) από τη Δ. Μεσόγειο και τη Θάλασσα του Μαρμαρά χωρίς να υπάρχουν δεδομένα για το Ιόνιο και το Αιγαίο Πέλαγος.

Η έλλειψη διαθέσιμων πληροφοριών σχετικά με τη βιολογία και τις φυλογενετικές σχέσεις των μελών της οικογένειας των Συγναθιδών κατά μήκος της ελληνικής ακτογραμμής αποτελεί ένα σημαντικό κενό στην επισκόπηση της βιολογίας και των φυλογενετικών τους σχέσεων καθώς το Ιόνιο και το Αιγαίο Πέλαγος: α) αποτελούνται από ένα ευρύ φάσμα οικοσυστημάτων με πληθώρα βιοτικών, αβιοτικών, φυσικοχημικών και άλλων περιβαλλοντικών παραγόντων, β) συνιστούν δύο διακριτές βιογεωγραφικές ζώνες γ) είναι σημαντικά οικοσυστήματα της Α Μεσογείου καθώς γειτνιάζουν με την Αδριατική Θάλασσα και τη Θάλασσα του Μαρμαρά.

Λαμβάνοντας υπόψη όλους τους παράγοντες που αναφέρθηκαν, σκοπός της παρούσας διδακτορικής διατριβής ήταν να μελετηθεί η βιολογία, η γενετική σύσταση, το φαινοτυπικό πρότυπο και η αναπαραγωγική συμπεριφορά των ειδών της οικογένειας των Συγναθιδών στον ελλαδικό χώρο. Η μελέτη πραγματοποιήθηκε στα δυο πιο άφθονα και εξαπλωμένα είδη της οικογένειας κατά μήκος της ελληνικής ακτογραμμής, τα οποία είναι τα *Syngnathus abaste*r και *Syngnathus typhle*.

Η μελέτη της βιολογίας των δυο ειδών πραγματοποιήθηκε με την εξέταση της σχετικής αφθονίας, της δομής πληθυσμών, της αναλογίας φύλων, της γεννητικής ωρίμανσης, των σχέσεων μήκους βάρους, του ηπατοσωματικού και του γοναδοσωματικού δείκτη. Τα άτομα των δύο ειδών που χρησιμοποιήθηκαν στη μελέτη συλλέχθηκαν σε δύο οικοσυστήματα του Β. Ιονίου, το Δρέπανο και το Νεοχώρι. Τα οικοσυστήματα αυτά απέχουν περίπου 80 χιλιόμετρα και διαφέρουν ως προς τον τύπο, την κάλυψη και την πυκνότητα της βλάστησης, την έκθεση τους στην ανοιχτή θάλασσα και τον κυματισμό, τον τύπο υποστρώματος και άλλους αβιοτικούς παράγοντες.

Στη παρούσα μελέτη εξετάστηκαν συνολικά 578 άτομα του είδους *S. abaster* (331 άτομα από το σταθμό του Δρεπάνου και 265 άτομα από το σταθμό του Νεοχωρίου). Η συνολική σχετική αφθονία των συλληφθέντων ατόμων δεν διέφερε στατιστικά σημαντικά μεταξύ των δύο οικοσυστημάτων. Όμως, σε κάθε σταθμό, διέφερε ο αριθμός των συλληφθέντων ατόμων ανά περίοδο καθώς συλλέχθηκαν λιγότερα άτομα τους χειμερινούς μήνες και περισσότερα την άνοιξη, το καλοκαίρι και το φθινόπωρο. Το γεγονός αυτό υποδηλώνει ένα μοντέλο εποχιακής μετανάστευσης, δηλαδή μετακίνηση των ατόμων σε μεγαλύτερα και προστατευμένα βάθη τους χειμερινούς μήνες. Ο σταθμός του Δρεπάνου αποτελούνταν από άτομα μήκους 28 (νεαρό άτομο) έως 253 (θηλυκό άτομο) χιλιοστών ενώ ο σταθμός του Νεοχωρίου από άτομα μήκους 23 (νεαρό άτομο) έως 238 (αρσενικό άτομο) χιλιοστών. Η κατά μήκος σύνθεση ώριμων και νεαρών ατόμων στους σταθμούς του Δρεπάνου και του Νεοχωρίου υπέδειξε δύο κλάσεις μεγεθών που αντιστοιχούν σε δύο ηλικιακές ομάδες. Η πρώτη ομάδα (0+) αποτελούνταν από νεαρά και ώριμα άτομα με ολικό μήκος μικρότερο από 120 χιλιοστά, ενώ η δεύτερη ομάδα (1+) αποτελούνταν μόνο από ώριμα άτομα ολικού μήκους μεγαλύτερου των 120 χιλιοστών. Λαμβάνοντας υπόψη την ύπαρξη αυτών των δύο ομάδων εκτιμάται ότι ο χρόνος ζωής του είδους ξεπερνά τους 12 μήνες και φτάνει μέχρι και τους 18-24 μήνες. Τα ώριμα άτομα (αρσενικά και θηλυκά) παρουσίασαν θετικό αλλομετρικό πρότυπο αύξησης και στους δύο σταθμούς ενώ τα νερά άτομα ισομετρικό. Στην αναλογία φύλων τα θηλυκά επικρατούσαν των αρσενικών ατόμων και στο αναπαραγωγικό δυναμικό τα θηλυκά επικρατούσαν των μη-κυοφορούντων αρσενικών ατόμων και στους δυο σταθμούς. Αυτή η κυριαρχία των θηλυκών ατόμων και στις δύο περιπτώσεις αποτελεί ένδειξη της αντιστροφής των φυλετικών ρόλων για το είδος. Η γεννητική ωρίμανση αρσενικών και θηλυκών ατόμων επιτελούνταν σε μήκος 70 χιλιοστών. Κατά τη διάρκεια της παρούσας μελέτης η αναπαραγωγική περίοδος διήρκησε από τις αρχές της άνοιξης (Μάρτιος- Απρίλιος) έως τα μέσα του φθινοπώρου (Οκτώβριος). Η διάρκεια της αναπαραγωγικής περιόδου εκτιμήθηκε από την ολοκλήρωση του σχηματισμού του εμβρυικού σάκου στα αρσενικά άτομα, την παρουσία κυοφορούντων αρσενικών ατόμων στα πεδία αναπαραγωγής και τις τιμές του γοναδοσωματικού δείκτη. Τέλος, στο σταθμό του Νεοχωρίου οι μεταβολές των τιμών του γοναδοσωματικού δείκτη αποτελούν ένδειξη συγχρονισμού της κύησης, φαινόμενο που δεν παρατηρείται στα άτομα του σταθμού του Δρεπάνου.

Για τη μελέτη των βιολογικών χαρακτηριστικών του είδους *S. typhle* εξετάστηκαν συνολικά 724 άτομα (287 άτομα από το σταθμό του Δρεπάνου και 437 άτομα από το

σταθμό του Νεοχωρίου). Η σχετική αφθονία των συλληφθέντων ατόμων δεν διέφερε στατιστικά σημαντικά μεταξύ των δύο οικοσυστημάτων. Όμως, σε κάθε σταθμό, διέφερε ο αριθμός των συλληφθέντων ατόμων ανά περίοδο καθώς συλλέχθηκαν λιγότερα άτομα τους χειμερινούς μήνες και περισσότερα τους υπόλοιπους. Το γεγονός αυτό μπορεί να οφείλεται στο φαινόμενο της χειμερινής μετανάστευσης. Ο σταθμός του Δρεπάνου αποτελούνταν από άτομα μήκους 33 (νεαρό άτομο) έως 243 (θηλυκό άτομο) χιλιοστών ενώ ο σταθμός του Νεοχωρίου από άτομα μήκους 25 (νεαρό άτομο) έως 188 (θηλυκό άτομο) χιλιοστών. Η κατά μήκος σύνθεση ώριμων και νεαρών ατόμων στους σταθμούς του Δρεπάνου και του Νεοχωρίου υπέδειξε τέσσερις κλάσεις μεγεθών που αντιστοιχούν σε τέσσερις ή και περισσότερες ηλιακές ομάδες. Η πρώτη ομάδα (0+) αποτελούνταν κυρίως από νεαρά άτομα και ελάχιστα ώριμα με ολικό μήκος μικρότερο από 80 χιλιοστά. Οι δεύτερη (1+), τρίτη (2+) και τέταρτη (> 2+) ομάδες αποτελούνταν μόνο από ώριμα άτομα με ολικό μήκος μικρότερο από 120 χιλιοστά, 160 χιλιοστά και μεγαλύτερο από 190 χιλιοστά αντίστοιχα. Τα ώριμα άτομα (αρσενικά και θηλυκά) παρουσίασαν θετικό αλλομετρικό πρότυπο αύξησης και στους δύο σταθμούς ενώ τα νερά άτομα ισομετρικό. Τα άτομα του είδους φτάνουν σε γεννητική ωριμότητα περίπου στα 75 χιλιοστά. Στη μελέτη του αναπαραγωγικού δυναμικού τα θηλυκά άτομα επικρατούσαν των μη-κυοφορούντων αρσενικών και στα δύο οικοσυστήματα. Αυτή η κυριαρχία των θηλυκών ατόμων ήταν αναμενόμενη καθώς το είδος παρουσιάζει αντιστροφή των φυλετικών ρόλων. Κατά τη διάρκεια της παρούσας μελέτης η αναπαραγωγική περίοδος διήρκεσε από τις αρχές της άνοιξης (Μάρτιος- Απρίλιος) έως τα μέσα του φθινοπώρου (Οκτώβριος). Η διάρκεια της εκτιμήθηκε από την ολοκλήρωση του σχηματισμού του εμβρυικού σάκου στα αρσενικά άτομα, την παρουσία κυοφορούντων αρσενικών ατόμων στα πεδία αναπαραγωγής και τις τιμές του γοναδοσωματικού δείκτη. Τέλος, στο σταθμό του Νεοχωρίου οι μεταβολές των τιμών του γοναδοσωματικού δείκτη αποτελούν ένδειξη συγχρονισμού της κύησης, φαινόμενο που δεν παρατηρείται στα άτομα του σταθμού του Δρεπάνου.

Η γενετική ανάλυση των δύο ειδών πραγματοποιήθηκε με τη χρήση δυο μοριακών δεικτών: της περιοχής ελέγχου του μιτοχονδριακού DNA και του πυρηνικού τόπου A1. Τα δείγματα συλλέχθηκαν το καλοκαίρι του 2010 από την παράκτια ζώνη της ηπειρωτικής Ελλάδας. Οι περιοχές από τις οποίες συλλέχθηκαν τα δείγματα είναι αντιπροσωπευτικές των κυριότερων βιογεωγραφικών ζωνών του Ιονίου και του Αιγαίου Πελάγους. Η ανάλυση και των δυο μοριακών δεικτών έδειξε ότι δεν υπάρχουν κοινοί απλότυποι ανάμεσα στα δύο υπό εξέταση είδη και ότι αποτελούν μονοφυλετικές ομάδες. Το γεγονός αυτό φαίνεται να αποκλείει τον υβριδισμό των ατόμων τους.

Τα αποτελέσματα της μελέτης του είδους *S. abaster* με βάση και τους δύο μοριακούς δείκτες φανέρωσαν, στη πλειοψηφία των περιπτώσεων, απουσία κοινών απλοτύπων μεταξύ των ατόμων του Ιονίου και Αιγαίου Πελάγους. Η έλλειψη κοινών απλοτύπων δηλώνει σε γενετικό επίπεδο την ύπαρξη δύο διακριτών πληθυσμών (Ιονίου και Αιγαίου Πελάγους). Γεωγραφικό όριο των δύο πληθυσμών πολύ πιθανό αποτελεί η Ν. Πελοπόννησος. Ενάντια στο γενικό πρότυπο, το φυλογενετικό δέντρο και το δίκτυο

απλοτύπων αποκάλυψε την παρουσία μικρού αριθμού κοινών απλοτύπων μεταξύ των ατόμων του Ιονίου (σταθμός Καλογριάς) και του Β.Κ. Αιγαίου Πελάγους.

Η παρατηρούμενη γενετική δομή του *S. abaster* κατά μήκος της ηπειρωτικής ακτογραμμής πολύ πιθανό να συνδέεται με τη παρουσία τουλάχιστον δύο καταφύγιων κατά τη διάρκεια των παγετώνιων-μεσοπαγετώνιων περιόδων: ένα για τον πληθυσμό του Αιγαίου και ένα δεύτερο για τον πληθυσμό του Ιονίου Πελάγους. Ορμώμενα από αυτά τα καταφύγια τα άτομα του είδους επανεποίκησαν το Ιόνιο και το Αιγαίο Πέλαγος. Παρά την γεωγραφική απομόνωση και τη μειωμένη δυνατότητα μετακίνησης των ατόμων του είδους (λόγω των οστέινων πλακών που καλύπτουν το σώμα των μελών της οικογένειας των Συγναθιδών και του βενθικού χαρακτήρα των ενήλικων και των νεαρών ατόμων του είδους), οι κοινοί απλότυποι που αναφέρθηκαν προηγουμένως μαρτυρούν την ύπαρξη μειωμένης γονιδιακής ροής ανάμεσα στους δύο πληθυσμούς. Το χρονικό διάστημα που πραγματοποιήθηκε αυτή η επαφή παραμένει άγνωστο και θα μπορούσε να είναι τόσο κατά την επανεποίκηση όσο και σε πιο πρόσφατη χρονολογική κλίμακα λόγω παθητικής μεταφοράς. Ωστόσο, η ένταση της γονιδιακής ροής δεν ήταν επαρκής για να οδηγήσει σε ομογενοποίηση των πληθυσμών του Ιονίου και του Αιγαίου Πελάγους.

Η μελέτη της γενετικής σύσταση του είδους *S. typhle* κατά μήκος της ελληνικής ακτογραμμής με βάση τους δυο μοριακούς δείκτες οδήγησε σε αντικρουόμενα αποτελέσματα. Η ύπαρξη γενετικής δομής παρατηρήθηκε μόνο στην ανάλυση του mtDNA, χωρίς όμως να αποδίδεται σε κάποιο γεωγραφικό όριο καθώς παρατηρήθηκαν κοινοί απλότυποι ανάμεσα σε απομακρυσμένους πληθυσμούς. Αυτό το πρότυπο θα μπορούσε να είναι το αποτέλεσμα μιας κοινής γονιδιακής δεξαμενής (επανεποίκηση του Ιονίου και του Αιγαίου Πελάγους από άτομα που αναζήτησαν ένα κοινό καταφύγιο στις Παγετώνιες περιόδους) ή / και το αποτέλεσμα παθητικής μεταφοράς των βενθοπελαγικών νεαρών ατόμων του είδους μέσω της επιπλέουσα βλάστησης. Στη περίπτωση της παθητικής μεταφοράς το φαινόμενο θα πρέπει να ήταν αρκετά έντονο ώστε να υπερνικήσει τη γεωγραφική απομόνωση και να οδηγήσει σε γενετική πανμιξία. Τα αντικρουόμενα αποτελέσματα της γενετικής σύστασης του είδους μπορεί να αποδοθούν στο διαφορετικό ρυθμούς εξέλιξης των δύο δεικτών και σε μια μεγαλύτερη δυνητικά ικανότητα διασποράς των θηλυκών ατόμων του είδους σε σχέση με τα αρσενικά.

Σε ευρωπαϊκό επίπεδο, η ανάλυση του μιτοχονδριακού DNA του είδους *S. typhle* έδειξε ότι οι ελληνικοί απλότυποι σχηματίζουν την ομάδα της Α. Μεσογείου, είναι διακριτοί από τους απλότυπους της Δ. Μεσογείου και παρουσιάζουν περιορισμένη συγγένεια με τους απλότυπους της Μαύρης Θάλασσας και της Θάλασσας του Μαρμαρά. Αντιθέτως τα αποτελέσματα της ανάλυσης του πυρηνικού DNA φανερώνουν ισχνότερη γενετική δομή στην οποία όμως οι βόρειοι πληθυσμοί του είδους είναι περισσότερο απομονωμένοι σε σχέση με τους νότιους (συμπεριλαμβανομένου και των ελληνικών απλοτύπων). Το παρατηρούμενο πρότυπο γενετικής σύστασης του είδους, τόσο σε τοπικό –ελληνικό- όσο και ευρωπαϊκό επίπεδο, φαίνεται να ενισχύει την υπόθεση ενός κοινού καταφυγίου στην Α Μεσόγειο κατά την Πλειστόκαινο περίοδο για τα άτομα του Ιόνιου και του Αιγαίου Πελάγους.

Το μορφολογικό πρότυπο των δύο ειδών διερευνήθηκε με τη μέθοδο της γεωμετρικής μορφομετρίας (πρωτόκολλο βασισμένο σε δεκαπέντε ορόσημα) και με τη σύγκριση επτά μεριστικών χαρακτήρων. Ανάμεσα στα δύο είδη δεν βρέθηκαν ενδιάμεσοι μορφότυποι γεγονός που υποδηλώνει πλήρη μορφομετρικό διαχωρισμό. Συγκεκριμένα, τα αποτελέσματα της ανάλυσης έδειξαν ότι τα άτομα του είδους *S. typhle* είχαν i) μεγαλύτερο ρύγχος, μεγαλύτερο κορμό και μικρότερο ουραίο μίσχο και ii) περισσότερους δακτυλίους στο τμήμα του σώματος τους που βρίσκεται μπροστά από το ραχιαίο πτερύγιο και λιγότερους δακτυλίους στον ουραίο μίσχο σε σχέση με τα άτομα του *S. abaster*. Η παρατηρούμενη διαφορά στο σχήμα του ρύγχους των δύο ειδών πιθανών να οφείλεται στη διαφορετική τροφική οικολογία τους. Συγκεκριμένα το μακρύ ρύγχος των ατόμων του *S. typhle* μπορεί να συνδέεται με την προτίμηση του είδους σε μεγάλα και ταχέως κινούμενα θηράματα. Αντίθετα το μικρό ρύγχος του *S. abaster* πιθανότατα σχετίζεται με προτίμηση του είδους σε μικρού μεγέθους λείες.

Η ανάλυση του φυλετικού διμορφισμού έδειξε ότι τα θηλυκά άτομα του *S. abaster* και του *S. typhle* είχαν πιο επίμηκες και παχύτερο ρύγχος και κυρίως κορμό ενώ είχαν λεπτότερο ουραίο μίσχο σε σχέση με τα αρσενικά. Το αποτέλεσμα αυτό θα μπορούσε να συνδέεται με το ρόλο των δύο φύλων την αναπαραγωγική περίοδο. Συγκεκριμένα, καθώς και τα δύο είδη επιδεικνύουν πατρική κύηση, τα αρσενικά άτομα χρειάζονται μεγαλύτερο ουραίο μίσχο σε σχέση με τα θηλυκά για να κυοφορήσουν τους απογόνους τους. Αντίθετα, τα θηλυκά άτομα χρειάζονται περισσότερο χώρο στη κοιλιακή περιοχή (κυρίως κορμός) για να παράγουν και να αποθηκεύουν όσο το δυνατό περισσότερα αυγά. Τέλος η παρατηρούμενη διαφορά στο μεγέθους του ρύγχος μπορεί να σχετίζεται με τις διαφορετικές διατροφικές συνήθειες των δύο φύλων κατά την αναπαραγωγική περίοδο.

Το ενδοειδικό φαινοτυπικό πρότυπο του *S. abaster* και του *S. typhle* έδειξε ότι και τα δύο είδη αποτελούνταν από δύο μορφομετρικά διακριτούς πληθυσμούς: του Αιγαίου και του Ιονίου Πελάγους. Αναφορικά με το είδος *S. typhle,* οι μορφομετρικά διακριτές ομάδες του Ιονίου και του Αιγαίου έρχονται σε αντίθεση με τα αποτελέσματα της γενετικής ανάλυσης. Καθώς οι μορφομετρικοί χαρακτήρες ελέγχονται μερικώς από τη γενετική σύσταση, το παρατηρούμενο μορφολογικό πρότυπο θα μπορούσε να είναι μια τοπική προσαρμογή στις διαφορετικές περιβαλλοντικές και φυσικοχημικές συνθήκες που επικρατούν στο Ιόνιο και στο Αιγαίο Πέλαγος.

Η μορφομετρική ανάλυση του *S. abaster* έδειξε ότι τα άτομα του Ιονίου είχαν πιο λεπτό σώμα και επίμηκες ρύγχος σε σύγκριση με του Αιγαίου Πελάγους. Ο πληθυσμός του Ιονίου Πελάγους χωρίζονταν περαιτέρω σε τρεις διακριτές ομάδες: i) Β. Ιόνιο-Κατάκολο, ii) Κοτύχι- Καλογριά και iii) Νεοχώρι (Αμβρακικός Κόλπος). Συγκεκριμένα, τα άτομα από την ομάδα του Κοτυχίου- Καλόγριας και της ομάδας του Νεοχωρίου είχαν πιο επίμηκες σώμα και παχύτερο κορμό από τα άτομα της ομάδας του Β. Ιονίου-Κατάκολου. Επίσης, τα άτομα από την ομάδα του Κοτυχίου- Καλογριάς είχαν μεγαλύτερη κεφαλική περιοχή, πιο επίμηκες ρύγχος και κύριο κορμό σε σύγκριση με τα άτομα των υπόλοιπων ομάδων. Ο πληθυσμός του Αιγαίου Πελάγους κατηγοριοποιούνταν περαιτέρω σε τρεις διακριτές ομάδες: i) Β.Δ. και Κ. Αιγαίο, ii) Β.Α. Αιγαίο και iii) Ν. Αιγαίο.

Συγκεκριμένα, άτομα της ομάδας του Β.Α. Αιγαίου είχαν μεγαλύτερη κεφαλική περιοχή, μικρότερα κύριο σώμα και λεπτότερο ουραίο μίσχο σε σύγκριση με τα άτομα των υπόλοιπων δύο ομάδων. Επίσης, τα άτομα από την ομάδα Ν. Αιγαίου είχαν πιο επίμηκες ρύγχος και το κυρίως κορμό σε σύγκριση με τα άτομα του Β. Αιγαίου.

Το μορφομετρικό πρότυπο τους είδους (διακριτοί πληθυσμοί Ιόνιου-Αιγαίου) συμφωνεί με τα αποτελέσματα της γενετικής ανάλυσης. Ωστόσο, η ομαδοποίηση στο εσωτερικό του Ιονίου και του Αιγαίου δεν ήταν ταυτόσημη με τις ομάδες που προέκυψαν στη γενετική ανάλυση του είδους. Δεδομένου ότι οι μορφομετρικοί χαρακτήρες ελέγχονται μερικώς από τη γενετική σύσταση, το παρατηρούμενο μορφολογικό πρότυπο θα μπορούσε να αποτελεί τοπική προσαρμογή στις διαφορετικές περιβαλλοντικές και φυσικοχημικές συνθήκες που επικρατούν στο Ιόνιο και στο Αιγαίο Πέλαγος, όπως και στη περίπτωση του *S. typhle*. Συγκεκριμένα, οι ομάδες εντός του Ιονίου και του Αιγαίου αντιστοιχούσαν σε διαφορετικούς τύπους οικοσυστημάτων.

Η διαδικασία της πατρικής κύησης και η γενετική αναπαραγωγική συμπεριφορά των *S. abaster* και *S. typhle* εξετάστηκαν σε κυοφορούντα αρσενικά άτομα τα οποία συλλέχτηκαν στο σταθμό του Δρεπάνου (Β. Ιόνιο Πέλαγος) στο πρώτο μισό της αναπαραγωγικής περιόδου του 2012. Τα κυοφορούντα αρσενικά και των δύο ειδών μεταφέρθηκαν στο εργαστήριο και τοποθετήθηκαν σε ειδικά διαμορφωμένα ενυδρεία μέχρι να ολοκληρωθεί η διαδικασία της κύησης και να απελευθερώσουν όλους τους απογόνους τους.

Έντεκα κυοφορούντα αρσενικά του είδους *S. abaster* και τρία του *S. typhle* γέννησαν υπό εργαστηριακές συνθήκες. Κατά τη διάρκεια της κύησης παρατηρήθηκαν αλλαγές στην υφή και στην εμφάνιση του εμβρυικού σάκου των δύο ειδών. Συγκεκριμένα, ο εμβρυικός σάκος από μια άκαμπτη δομή παρόμοιου χρώματος με το υπόλοιπο σώμα (αρχικά στάδια κύησης) γινόταν πιο μαλακός και σκουρόχρωμος στα τελικά στάδια της κύησης. Την ημέρα του τοκετού, οι αρσενικοί γεννήτορες εμφάνιζαν μειωμένη κινητικότητα και ο εμβρυικός σάκος ήταν έτοιμος να ανοίξει. Στη πλειοψηφία τους οι αρσενικοί γεννήτορες (ανεξαρτήτως είδους) απελευθέρωσαν τους απογόνους τους τη νύχτα ή νωρίς το πρωί σε διαδοχικές σποραδικές παρτίδες με απότομες κυματιστές κινήσεις του σώματός τους. Ο τοκετός διαρκούσε περίπου 24 ώρες. Οι απόγονοι που απελευθερώνονταν ήταν πλήρως σχηματισμένοι και έμοιαζαν στα ώριμα άτομα κάθε είδους. Συγκεκριμένα οι απόγονοι του *S. abaster* (43 απόγονοι κατά μέσο όρο ανά γεννήτορα) εντοπίζονταν συνήθως στον πυθμένα του ενυδρείου, όπου κολυμπούσαν ή κρύβονταν στη άμμο (βενθική συμπεριφορά). Αντιθέτως, τα νεογέννητα άτομα τους *S. typhle* (43 απόγονοι κατά μέσο όρο ανά γεννήτορα) εντοπίζονταν να κολυμπούν κοντά στην επιφάνεια του ενυδρείου (βενθοπελαγική συμπεριφορά). Το ολικό μήκος και βάρος καθώς και ο αριθμός των ακτίνων του ραχιαίου πτερυγίου διέφερε μεταξύ των απογόνων του κάθε είδους. Επίσης, παρατηρήθηκε χρωματική διαβάθμιση μεταξύ των απογόνων από μια ελαφριά απόχρωση (ανοιχτό πράσινο ή καφέ), σε μια πιο σκοτεινή (σκούρο πράσινο ή καφέ) ή ακόμη και μαύρη. Ωστόσο, γενικά, δεν καταγράφηκαν στατιστικά σημαντικές

συσχετίσεις του ολικού μήκους, βάρους και του αριθμού των ακτίνων του ραχιαίου πτερυγίου με τα χρωματικά πρότυπα των απογόνων.

Για την εκτίμηση της γενετικής αναπαραγωγικής συμπεριφοράς των ειδών *S. abaster* και *S. typhle* μελετήθηκαν οι μικροδορυφορικοί τόποι S. abas3, S.abas4, S.abas7 και S.abas9. Οκτώ κυοφορούντα αρσενικά του είδους *S. abaster*, ένα υποσύνολο των απογόνων τους (N = 249) και τριάντα τεσσάρα άτομα (θηλυκά και αρσενικά) από σταθμό του Δρέπανου γενοτυπήθηκαν επιτυχώς σε κάθε ένα από τους τέσσερις τόπους. Η πατρότητα όλων των απογόνων κάθε αρσενικού γεννήτορα πιστοποιήθηκε με χρήση του λογισμικό Colony Τα οκτώ κυοφορούντα αρσενικά άτομα που αναλύθηκαν δέχτηκαν συνολικά αυγά από εικοσιοκτώ θηλυκά. Συγκεκριμένα σε κάθε αρσενικό γεννήτορα εναποθέτονταν αυγά από τρία έως έξι θηλυκά άτομα. Ο αριθμός των αυγών που εναπόθετε κάθε θηλυκό στον εμβρυικό σάκο του αρσενικού κυμαίνονταν από ένα έως εικοσιτέσσερα. Μεταξύ των απογόνων των θηλυκών γεννητόρων στατιστικά σημαντικές διαφορές παρατηρήθηκαν στο ολικό μήκος και τον αριθμό των ακτίνων του ραχιαίου πτερυγίου.

Τρία κυοφορούντα αρσενικά του είδους *S. typhle*, ένα υποσύνολο των απογόνων τους (N = 78) και δεκαοκτώ άτομα (θηλυκά και αρσενικά) από σταθμό του Δρέπανου γενοτυπήθηκαν επιτυχώς σε κάθε ένα από τους τέσσερις μικροδορυφορικούς τόπους. Η πατρότητα όλων των απογόνων κάθε αρσενικού γεννήτορα πιστοποιήθηκε με χρήση του λογισμικό Colony. Τα τρία κυοφορούντα αρσενικά άτομα που αναλύθηκαν δέχτηκαν συνολικά αυγά από επτά θηλυκά. Δύο από τα τρία αρσενικά δέχτηκαν αυγά από περισσότερα του ενός θηλυκά (ως και τρία), ενώ ένα αρσενικό έλαβε αυγά μόνο από ένα θηλυκό. Ο αριθμός των αυγών που εναπόθετε κάθε θηλυκό στον εμβρυικό σάκο του αρσενικού κυμαίνονταν από ένα έως είκοσι εννιά. Από τα αποτελέσματα της παρούσας μελέτης δεν προκύπτουν θηλυκοί γεννήτορες που να έχουν εναποθέσει τα αυγά τους σε περισσότερο του ενός αρσενικά.

Για να συγκριθεί το μορφολογικό πρότυπο των νεογέννητων ατόμων ($1^{ης}$ ημέρας) των ειδών *S. abaster* και *S. typhle* αναλύθηκαν 278 και 92 άτομα από κάθε είδος αντίστοιχα. Το γεωμετρικό μορφομετρικό πρωτόκολλο που εφαρμόστηκε ήταν ίδιο με αυτό που χρησιμοποιήθηκε στη μορφομετρική ανάλυση των ενήλικων ατόμων των δύο ειδών. Η Γραμμική Διαχωριστική Ανάλυση (LDA). έδειξε μια στατιστικά σημαντική διαφοροποίηση των νεογέννητων ατόμων των δύο ειδών, τα οποία δημιουργούν δυο διακριτές ομάδες: i) το είδος *S. typhle* και ii) το είδος *S. abaster*. Συγκεκριμένα τα άτομα του είδους *S. typhle* είχαν πιο επίμηκες ρύγχος, μακρόστενο κύριο κορμό, ενώ ο ουραίος τους μίσχος ήταν μικρότερος σε σύγκριση με του *S. abaster*. Οι διαφορές αυτές συμφωνούν και με τα μορφομετρικά χαρακτηριστικά που διαχωρίζουν τα ώριμα άτομα των δύο ειδών. Το γεγονός ότι οι μορφομετρικές διαφορές των ενήλικων ατόμων των *S. abaster* και *S. typhle* είναι ανιχνεύσιμες ακόμα και στα νεογέννητα άτομα, δηλώνει ότι απόγονοι των δύο ειδών κατά την απελευθέρωση τους από τον εμβρυικό σάκο είναι πλήρως ανεπτυγμένα άτομα τα οποία παρουσιάζουν στενή ομοιότητα με τα ενήλικα άτομα του είδους τους.

Εν κατακλείδι, η συγκριτική μελέτη των δυο υπό εξέταση ειδών φανέρωσε ομοιότητες και διαφορές. Σχετικά με τη βιολογία των *S. abaster* και *S. typhle*, και τα δύο είδη έχουν δημιουργήσει στο Β. Ιόνιο εδραιωμένους πληθυσμούς με εκτεταμένη αναπαραγωγική περίοδο. Η μελέτη της γενετική αναπαραγωγική συμπεριφορά επιβεβαίωσε τον πολυγύνανδρο χαρακτήρα του *S. abaster* και φανέρωσε τον μεγαλύτερο αριθμό θηλυκών γεννητόρων που έχει καταγραφεί στις μέχρι τώρα μελέτες. Ο πολύγυνος χαρακτήρας του *S. typhle* χρήζει περεταίρω επιβεβαίωση με μελέτη μεγαλύτερου αριθμού αρσενικών γεννητόρων. Οι φυλογενετικές σχέσεις και το μορφομετρικό πρότυπο των δύο ειδών ήταν πλήρως διακριτά γεγονός που φανερώνει απουσία φαινομένου υβριδισμού. Παρόλα αυτά τα δύο είδη παρουσίασαν διαφορετικό βαθμό γενετικής δομής. Το γεγονός αυτό φανερώνει την επίδραση ιστορικών (περιβαλλοντικά και υδρογεωλογικά φαινόμενα που διαδραματίστηκαν στη Πλειστόκαινο περίοδο) και πιο σύγχρονων (στοιχεία βιολογίας) επιρροών στο βαθμό διαφοροποίησης των δύο ειδών.

Chapter 1. General introduction

1.1. Biology of syngnathids

1.1.1. Life history traits of syngnathids

Marine shallow coastal ecosystems are highly productive and susceptible to seasonal changes and physical disturbance (Planes et al. 2000; De Raedemaecker et al. 2010). In these habitats, numerous species seek food (Ornellas and Coutinho 1998; Hindell et al. 2000; Kendrick and Hyndes 2005) and sheltered reproductive/nursery grounds (juveniles of several migratory species), while only few are resident (García-Rubies and Macpherson 1995; Nagelkerken et al.1 2000; Cocheret de la Morinière et al. 2002; Lloret et al. 2002). The latter are usually characterized by small size (e.g. *Atherina boyeri* and *Aphanius fasciatus*), short life cycle (e.g. *A. boyeri* and *A. fasciatus*), one or few reproductive seasons (e.g. *A. fasciatus, Syngnathus abaster*), and increased parental care (*Pomatoschistus minutus, S. abaster* and *Syngnathus typhle*). (Leonardos and Sinis 1999; 2000; Lindström and Hellström 1993; Vincent et al. 1995).

Seahorses (genus *Hippocampus*), pipefishes (e.g. genera *Syngnathus* and *Nerophis*), seadragons (*Phycodurus eques, Phyllopteryx taeniolatus*) and pipehorses (e.g. genus *Solegnathus*) (Figure 1.1) are members of the family of Syngnathidae and are typical resident species of temperate and tropical marine, freshwater and brackish shallow coastal ecosystems across the world (Pollard 1984; Hindell et al. 2000; Kendrick and Hyndes 2005). Despite their worldwide distributional range, they are generally associated with sea grass beds (Erzini et al. 2002), which provide them with a sheltered environment to live and reproduce (Teixeira and Vieira 1995; Vincent et al. 1995). Some syngnathids are abundant in many habitat types, whereas other species are associated only with specialized ones (Foster and Vincent 2004). Their greatest diversity occurs in the Indo-Pacific where most of the known species are found (Dawson 1986). Shokri et al. (2009) has provided evidence of *"a spatial correlation between the presence of syngnathids and other fish taxa in littoral seagrass habitats and thus their utility as flagship species*".

Syngnathids exhibit a high diversity of morphological forms (size, body shape, color pattern, ornaments, body posture, fin arrangement and snout phenotype), but are generally characterized by elongated snouts, fused jaws, absence of pelvic fins and thick plates of bony armor covering their bodies (Dawson 1986; McEachran and Fechhelm 1998; Lourie et al. 1999). The armor provides protection against predator but also reduces the swimming ability and thus the dispersal ability of all members of the family. In temperate waters during winter months- once the shallow-water vegetation dies- species migrate into deeper waters (Lazzari and Able 1990; Franzoi et al. 1993; Teixeira 1995; Bolland and Boeticher 2005).

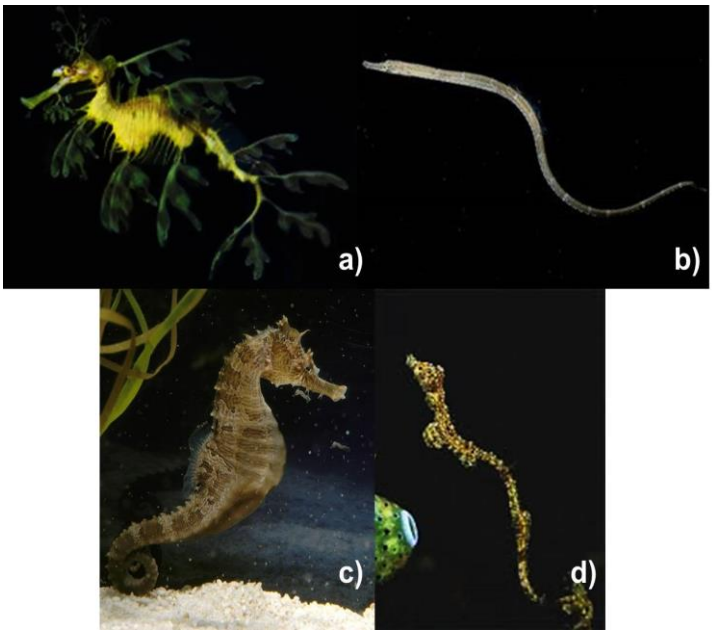

Figure 1.1. Members of the family of Syngnathidae: a) seadragon, b) pipefish, c) seahorse, d) pipehorse.
Εικόνα 1.1. Μέλη της οικογένειας των Συγναθιδών a) θαλάσσιος δράκος, b) σακοράφα, c) ιππόκαμπος, d) ταινιόμορφος ιππόκαμπος.

Their lifespan ranges from one to five years, even though recent studies have indicated that in some species this may reach up to seven or eight years (Franzoi et al. 1993; Lockyear et al. 1997; Barrows et al. 2009; Miersch 2012). In most studies, age estimates derive from length frequency distribution and most recently, from otolith examinations (Campbell and Able 1998, Barrows et al. 2009, Bolland and Boeticher 2005, Miersch 2012). During the first months of their life, syngnathids exhibit a rapid growth rate, which slows down after sexual maturity (Fritzsche 1980; Woods 2005). Male growth is negligible during brood incubation, creating a trade-off between maturation time and adult body size (Svensson 1988). Syngntahids' growth depends on temperature (James and Woods 2001, Takahashi et al. 2003) while mortality declines as body size increases (Houde 1997, Sanchez-Camara et al. 2005). Juveniles are facing the highest mortality rates probably due to predation by many fish (even by adult syngnathids) and invertebrates (Franzoi et al. 1993; Miersch 2012).

Syngnathids may reproduce all year long (Barrows et al. 2009; Ishihara and Tachihara 2009) or have a well-defined period ranging from three to nine months (all year long except for winter months) depending on water temperature and latitude (Campbell and Able 1998; Lyons and Dunne 2003; Takahashi et al. 2003; Foster and Vincent 2004; Bolland and Boeticher 2005). In the latter case, northern populations have a shorter mating and brooding season than southern ones (Campbell and Able 1998). Prior to mating, most species are engaged in an elaborate courtship behavior that includes a prenuptial dance (Vincent et al. 1995; Silva et al. 2006b). The brood size and the size of the young vary among species (e.g. Teixeira 1995; Franzoi et al. 1993; Silva et al. 2006a). *Hippocampus ingens* species have the largest brood clutches recorded so far (up to 2000 embryos)

(Woods 2005). However, typical brood size of most syngnathids is much smaller (around 200 embryos) (Foster and Vincent 2004; Sanchez-Camara et al. 2005; Woods 2005; Ishihara and Tachihara 2009).

The population structure of syngnathids may compose of equally sized males and females in similar ratios –seahorses-, to sexually sized dimorphic individuals with biased sex ratio –pipefishes (Steffe et al. 1989; Franzoi et al. 1993; Curtis and Vincent 2006; Barrows et al. 2009). In the latter case, the population is usually female biased (Franzoi et al. 1993; Bolland and Boeticher 2005; Monteiro et al. 2006; Barrows et al. 2009).

The feeding ecology of syngnathids is, also, well studied. Syngnathids as ambush predators stay still, while waiting for their prey to approach their mouth (Muller 1987; Foster and Vincent 2004; Leysen 2011). Expansion of their snout generates a fast water flow that carries the prey into the mouth- pivot feeding strategy (de Lussanet and Muller 2007). They are known to consume mainly nematodes and small crustaceans such as amphipods and copepods (Foster and Vincent 2004; Kendrick and Hyndes 2005; Castro et al. 2008). In some species ontogenetic changes in feeding habits occur, concerning the type, proportion and size of prey. For example in *Syngnathus typhle*, diet changes progressively from Copepods to Mysidacea and to small size Caridea and Gobiidae as body size grows (Franzoi et al. 1993; Kendrick and Hyndes 2005; Oliveira et al. 2007). On the contrary, other species, such as *Syngnathus abaster* and *Syngnathus acus*, demonstrate moderate diet succession with a preference on small-sized copepods hidden in the vegetation (Franzoi et al. 2004).

However, the biological characteristics that have drawn most attention on syngnathids over the last decades are male pregnancy, sex role reversal and their complex mating system (e.g. Vincent et al. 1995; Monteiro et al. 2002; Silva et al. 2006a; Rosenqvist and Berglund 2011). These characteristics are the main core of the present thesis and are analyzed in the following pages.

1.1.2. Male pregnancy and parental care of syngnathids

Male pregnancy is probably the most interesting aspect of syngnathids biology. Females deposit their eggs on a ventral incubating structure either on the tail (Urophori) or the trunk (Gastrophori) of the male body. The complexity of this structure varies (Herald 1959; Wilson et al. 2001) and eggs can be placed in a:

- brooding area, where they are completely unprotected (genera *Nerophis* and *Entelurus*),
- brooding area separated by compartments formed by membranous inner folds (genera *Solegnathus* and *Doryrhamphus*),
- structured pouch where they are protected by pouch plates (extension of body plates) (genus *Oostethus*),

- structured pouch where they are partially or fully protected by fleshy bilateral folds (genus *Syngnathus*),
- completely enclosed pouch, which opens through a slit or pore when the offspring are released (genus *Hippocampus*).

The development of the syngnathid brood pouch could have its roots in the parental care of Gasterosteidae which build nests and guard their offspring (Baylis 1981; Wilson et al. 2001). However, within their nests, Gasterosteoids eggs are threatened by predation and sneaky males who aim to fertilize them (Wootton 1984). Under these circumstances of high nest predation, males of a hypothetical pre-pipefish ancestor may have attached some eggs onto themselves, thereby securing the survival of at least a few young (Baylis 1981; Wilson et al. 2001). This response may have been a primitive form of brood pouch (Berglund and Rosenvist 2013). Its evolution (from simple to complex forms) in syngnathids presumably worked as a "safety net" against their enemies, increasing their reproductive success and ensuring males that they are the true fathers of their offspring (Jones and Avise 1997; Jones et al. 1999; McCoy et al. 2001). Seahorses have the most complex pouch structure and are subjected to the most significant physiological changes during embryo incubation (Foster and Vincent 2004).

Although the physiology of the syngnathids brood pouch has been the subject of many studies, many disputes exist on its function (e.g. Carcupino et al. 1997; Wilson et al. 2001; Paczolt and Jones 2010). In both Urophori and Gastrophori, females transfer their yolk-rich eggs (Foster and Vincent 2004) to male pouch and fertilization is achieved during egg transfer (Watanabe et al. 2000; VanLook et al. 2007). The enclosed brood pouch seals off the eggs and the embryos from external conditions (Svensson 1988).

In species with complex structure, the fundamental changes in the morphology of the pouch during egg incubation clearly indicate that its role is far more than mere protection (Carcupino et al. 2002). For instance, in the pouch of several pipefish species, mitochondrial-rich cells (MR) have been identified during and shortly after incubation (Carcupino et al. 2002; Watanabe et al. 1999). MRs play an important role in adult fish osmoregulation (Carcupino et al. 1997; Partridge et al. 2007; Ripley and Foran 2009). Moreover, the blood vessels in the connective tissue of the pouch increase after implantation and gestation enabling males to oxygenate the embryos. (Carcupino et al. 2002; Stolting and Wilson 2007; Ripley et al. 2010). Also, important nutrients for the growth of the embryos are believed to be transported through the brood pouch (Partridge et al. 2007; Paczolt and Jones 2010; Sagebakken et al. 2010). Finally, parents contribute calcium to their embryos via the brood pouch, thus assisting in the skeletal development of their offspring (Linton and Soloff 1964; Carcupino et al. 2002). Carcupino et al. (2002) showed that "*the interactions between male body and the developing embryos are inversely proportional to the degree of egg exposure to the external environment and directly proportional to the anatomical complexity of the pouch*".

During parturition as the embryos are released the pseudo-placenta is detached, too. The released offspring assume position in the water column and fathers are no longer responsible for them (Ripley and Foran 2006). The brood pouch returns to the non-reproductive state once the reproductive period is over (Laksanawimol et al. 2006).

Finally, as already mentioned, the degree of exposure to ambient water and parental care are negatively correlated. Thus, offspring released from marsupium lacking species are smaller, less developed and undergo a larval stage compared to the fully formed offspring of the more evolved pouch type (Monteiro et al. 2003, Silva et al. 2006a).

1.1.3. Sex role reversal of syngnathids

Syngnathids are among the most studied organisms in the light of sexual selection and sex role patterns (Berglund et al. 1986; Steffe et al. 1989; Berglund 1991; Vincent et al. 1992). In some syngnathid species, sex-role patterns have been evaluated directly by estimating the bias in the operational sex ratio (OSR)[1] or the potential reproductive rate of males versus females (PRR)[2] (Vincent et al. 1992; Masonjones and Lewis 2000). At the same time, qualitative traits i.e. the degree of secondary sexual traits can be indirect evidence on the force of sexual selection (Berglund et al. 1996; Monteiro et al. 2002). In such case, the brighter colored gender is the one towards which operational sex ratio will lean to (Clutton-Brock and Vincent 1991).

The occurrence of male parental care has long misled the scientific community into thinking that all syngnathid species are sex-role reversed (Trivers 1972, 1985). However, recent studies indicate that, although paternal care is evident in all syngnathids, either sex may compete for mates. Hence, not all syngnathids are sex-role reversed (Vincent et al. 1992). Most pipefishes (*Syngnathus* sp. and *Nerophis* sp.) are sex role reversed, while seahorses exhibit conventional patterns (Vincent et al. 1992). Among the two species of seadragons both reversed and traditional patterns are observed (Wilson et al. 2003; Sanchez-Camara et al. 2005). Pipehorses are the least studied group, leaving no room for deduction and generalizations (Takahashi et al. 2003).

The intensity of sexual selection differs among pipefish, as it is implied by the degree of sexual dimorphism (Berglund et al 1986). For example, as regards the two sex-role reversed species *Nerophis ophidion* and *Syngnathus typhle*, the former's females are more active in courtship, are much larger than males and have evolved sexual characteristics consisting of a permanent blue coloring along their sides and a ventral skin

[1] OSR is the ratio of sexually competing males that are ready to mate to sexually competing females that are ready to mate.

[2] PRR is the maximum number of independent offspring that parents can produce per unit time

fold that develops during the breeding season and enlarge the female's appearance (Berglund et al. 1986). On the other hand, in *S. typhle* species, the two sexes are more similar in size and equally active during courtship while females display only a temporary color pattern (Berglund et al. 1986).

Sex role reversal arose independently from the degree of brood pouch development or parental care (Berglund et al. 1986; Vincent et al. 1992; Wilson et al. 2001; 2003; Berglund and Rosenqvist 2003). This is verified by the fact that, even though *N. ophidion* has a simple pouch it exhibits sex role reversed patterns. At the same time seahorses exhibit conditional sex roles, despite the more complicated pouch (Vincent et al. 1994; Berglund and Rosenqist 2003).

The factor mostly affecting sex role reversal and sexual selection in syngnathids is water temperature. Since water temperature is negatively correlated to latitude, male pipefish inhabiting colder waters are expected to exhibit a higher degree of selectivity in their choice of mates, while female-female competition grows stronger (Ahnesjo 1995; Rispoli and Wilson 2008; Mobley and Jones 2009).

1.1.4. Genetic mating system of syngnathids

As already mentioned syngnathids may exhibit conventional or reversed sex roles and court with only one partner (social monogamy) or multiple partners (social polygamy) (e.g. Vincent et al. 1995; Avise et al. 2002; Silva et al. 2006b). Vincent et al. (1992) and Wilson et al. (2003) showed an association between the mating and the sex role patterns in this family: "*polygamous species are sex role reversed, whereas monogamous species show conventional sex roles*" (Figure 1.2). However, mating patterns and sexual selection are uncorrelated to pouch complexity (Avise et al. 2002; Wilson et al. 2003) (Figure 1.3).

Studies on the genetic mating system of *Syngnathus* sp. have revealed diverse patterns from extreme polyandry accompanied with strongly sexual dimorphism (*Syngnathus scovelli*), to polygynandry with intermediate levels of sexual dimorphism (*Syngnathus abaster*, *Syngnathus typhle* and *Syngnathus floridae*) (Jones and Avise 1997, 2001; Jones et al. 1999; Jones and Avise 2001; Mobley and Jones 2007; Hubner et al. 2013). On the contrary, all species of *Hippocampus* so far examined, appear to be monogamous (Jones et al. 1998, Kvarnemo et al. 2000, Wilson and Martin-Smith 2007) and often maintain long-term pair bonds during the same (Kvarnemo et al. 2000) or consecutive (Harasti et al. 2012) breeding seasons.

Differences in mating patterns and intensity of sexual selection exist across different populations due to differences in demography (Mobley and Jones 2007; Rispoli and Wilson 2008), temperature (Rispoli and Wilson 2008; Mobley and Jones 2007, 2009;

Wilson 2009; Monteiro and Lyons 2012) or habitat (Mobley and Jones 2009). However, in most cases the mating system is described only in one population from each species.

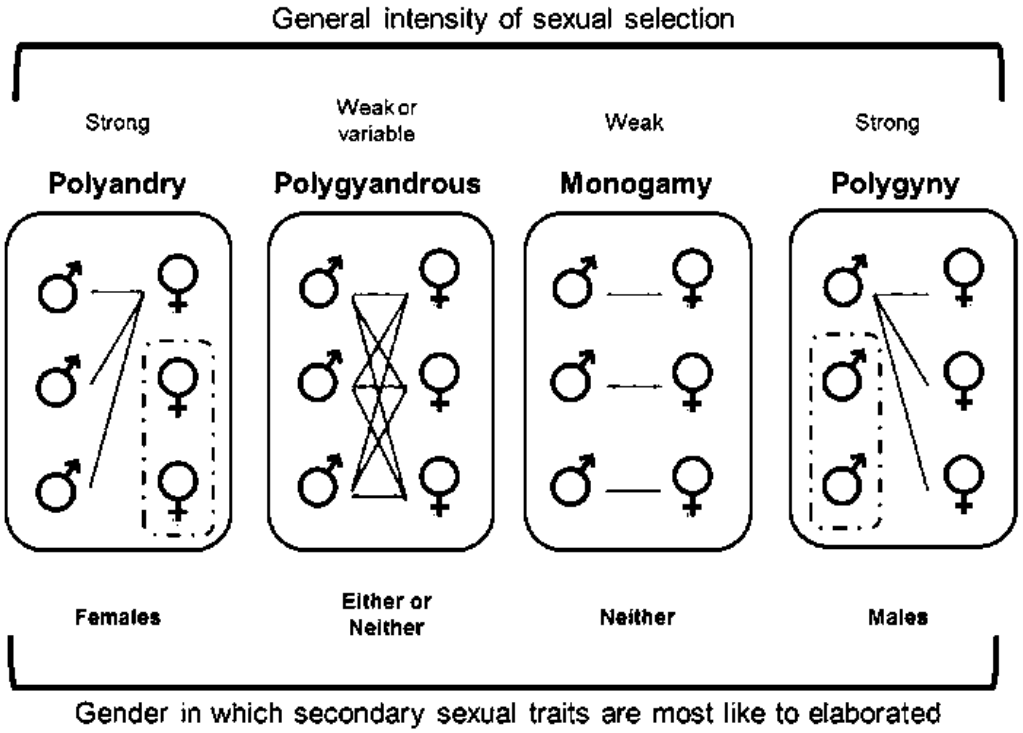

Figure 1.2.Different mating systems found in wild populations. The possible theoretical relation between mating systems and the intensity of sexual selection is shown (on the top), together with which sex is most likely to have elaborated secondary sexual characters (on the bottom); (*Continued lines*, mating partners producing offspring; *dotted lines* excluded individuals) (after Cunha 2012).

Εικόνα 1.2. Τύποι αναπαραγωγικής συμπεριφοράς. Η ένταση της σεξουαλικής επιλογής σε κάθε τύπο υποδεικνύεται στο πάνω τμήμα της εικόνας. Στο κάτω τμήμα υποδεικνύεται το φύλο στο οποίο εμφανίζονται πιο έντονα τα δευτερογενή σεξουαλικά χαρακτηριστικά (*Συνεχόμενες γραμμές,* πιθανοί συνδυασμοί των ατόμων σε κάθε τύπο συστήματος, *Διακεκομμένες γραμμές*, συνδυασμοί που αποκλείονται) (σύμφωνα με Cunha 2012).

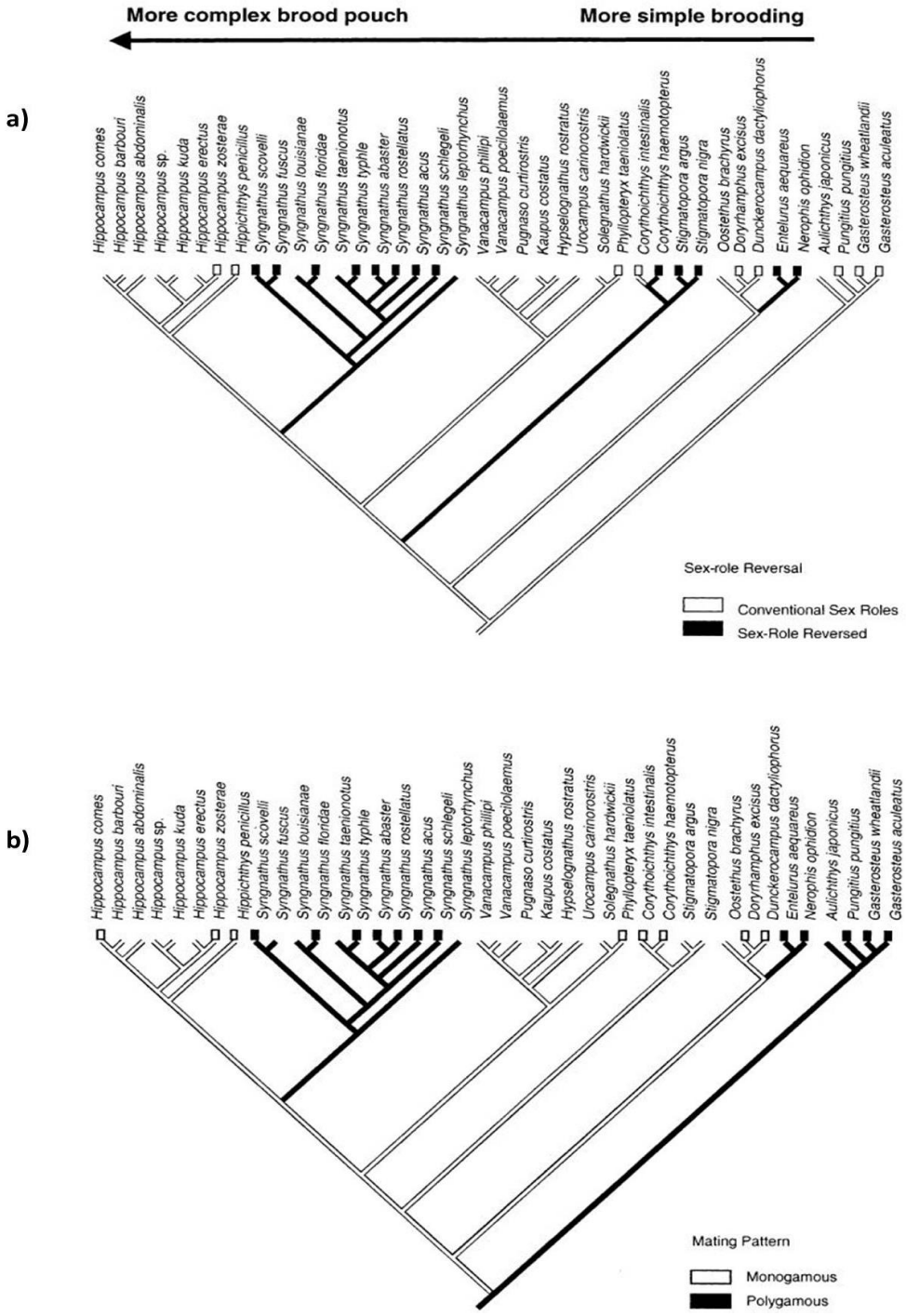

Figure 1.3. Relationship of brood pouch complexity with a) sex role and (b) mating patterns in the family of Syngnathidae (after Wilson et al. 2003).

Εικόνα 1.3. Σχέση του βαθμού πολυπλοκότητας του εμβρυικού σάκου με a) τη σεξουαλική συμπεριφορά και b) το αναπαραγωγικό σύστημα της οικογένειας των Συγναθιδών (σύμφωνα με Wilson και συν. 2003)

1.2. Phylogenetic relationships of syngnathids

1.2.1. The family of Syngnathidae

The family of Syngnathidae belongs to the order of Syngnathiformes. The evolutionary history of this order is under debate due to the high morphological diversity of its members (e.g. Orr 1995; Keivany and Nelson 2006; Wilson and Orr 2011). Molecular studies attempted to resolve this problem but there are still missing parts of the puzzle (e.g. Chen et al. 2003; Kawahara et al. 2008; Li et al. 2009). Nevertheless, both morphological and molecular studies showed that the family of Solenostomidae (ghost pipefishes) is the sister group of the Syngnathidae and Pegasidae (seamoths) (Wilson and Rouse 2010; Wilson and Orr 2011). The fact that both gost pipefishes and seamoths exhibit parental care (Herold and Clark 1993; Orr and Fritzsche 1993; Sado and Kimura 2006) indicates that the brooding trait may have preexisted male pregnancy (Wilson and Orr 2011).

Even though the order's phylogenetic analysis is ambiguous, the evolutionary relationships within syngnathids are (or at least thought to be) more clear (Wilson and Rouse 2010; Wilson and Orr 2011). The family consists of four subfamilies; Doryrhampinae, Nerophinae, Hippocampinae and Syngnathinae (Kaup 1856, according to Nelson 2006). The location and complexity of the male brood pouch further separates syngnathids into Gastrophori (abdominal-bearing) and Urophori (tail-bearing) (Duncker 1912; 1915, according to Wilson et al. 2001) and played a key role in the evolutionary history of the family (Herald 1959, Wilson et al. 2001).

The segregation of syngnathids into Gastrophori and Urophori occurred early in the evolution of the family (Herald 1959, Wilson et al. 2001). Both lineages evolved independently, with remarkable increase in pouch complexity observed in contemporary species (Wilson et al. 2001; 2003). The results of the genetic analysis are also supported by the karyotypic study of Viturri et al. (1998) who proposed total-genome duplication in the Gastrophori lineage. However, two phylogenetic analyses- including a wider variety of species- have questioned the validity of the family taxonomy and the Urophori and Gastrophori separation (Lourie and Randall 2003; Wilson and Rouse 2010). Therefore, it is obvious that a deeper level phylogenetic study is needed in order to shed light and settle the dispute about the taxonomy of the family of Syngnathidae.

1.2.2. Pipefishes and seahorses

As already mentioned, the two most proliferate and well-studied genera in the family of Syngnathidae are *Syngnathus* (common name pipefish) (e.g. Mobley and Jones 2007; Wilson and Veraguth 2010; Sanna et al. 2013a; Mwale et al. 2013) and *Hippocampus* (common name seahorse) (Casey et al. 2004; Teske et al. 2004; 2007; Woodall et al. 2011).

The cosmopolitan *Syngnathus* genus (found in marine, freshwater and estuarine habitats all over the world) has a Pacific origin (Wilson et al. 2001). The two most basal lineages in the *Syngnathus* phylogeny, *Syngnathus exilis* and *Syngnathus leptorhynchus* are restricted to the Eastern Pacific (coast of North America). The rest of the species are divided into three major clades: a) the Atlantic coast group (*Syngnathus fuscus* and *Syngnathus scovelli*), b) the Atlantic coast and Caribbean group and c) the western Pacific (*Syngnathus schlegeli)* and the European pipefishes (Wilson et al. 2001). Seahorses have an Australasian (Indo-Pacific) origin as the most basal (*Hippocampus abdominalis*) and second most basal (*Hippocampus breviceps*) lineages occur in the Indo-West Pacific and in Australia respectively (Casey et al. 2004; Teske et al. 2004). According to the most dominant hypothesis, the main group of seahorses is classified into three major clades, two with Indo-Pacific affinities and one with a circumglobal distribution (Teske et al. 2004; Wilson and Orr 2011).

1.3. Syngnathids along the European coastline

The European coastline is highly diverse as it consists of two oceanic (Arctic and Eastern Atlantic Ocean) and five marine ecosystems (Baltic, North, Mediterranean, Black and Caspian Seas). The last major event that caused dramatic shifts on the European coastline and shaped its population structure was the repeated glacial– interglacial cycles of the Pleistocene period (Svendsen et al. 2004). The northern and central coasts were severely affected as they were fully glaciated (Bjork 1995; Lambeck 1995). Even though southern Europe remained unglaciated, its coastline retreated and Mediterranean and Black Sea were separated (Mudie et al. 2002). The reconnection of the two seas affected and shaped the local biodiversity (Mudie et al. 2002; Ryan et al. 2003).

The bony armor that covers the body of the syngnathids makes them resilient to temperature, salinity and other major environmental fluctuations (Hilomen-Garcia et al. 2003; Wilson and Veraguth 2010). Therefore as revealed by fossil records and genetic analyses, syngnathids persisted both the Messinian salinity crisis and the Pleistocene glaciation (Wilson and Veraguth 2010; Alaya et al. 2011; Sanna et al. 2013a; Woodall et al. 2011). To endure such major events syngnathids took refugium in protected areas and then recolonized old and new habitats (Lourie and Vincent 2004; Wilson and Veraguth 2010; Alaya et al. 2011; Sanna et al. 2013a; Woodall et al. 2011).

In the present, there are 16 species of the family of Syngnathidae across the European coastline (Figure 1.4) (Dawson 1986): *Entelurus aequoraeus* (Linnaeus 1758), *Hippocampus hippocampus* (Linnaeus 1758), *Hippocampus guttulatus* Curvier 1829, *Minyichthys sentus* Dawson 1982, *Nerophis lumbriciformis* (Jenyns 1835), *Nerophis maculatus* Rafinesque 1810, *Nerophis ophidion* (Linnaeus 1758), *Syngnathus abaster* Risso 1826, *Syngnathus acus* (Linnaeus 1758), *Syngnathus phlegon* Risso 1826, *Syngnathus rostellatus* Nilsson 1855, *Syngnathus schmidti* Popov 1928, *Syngnathus taenionutus* Canestrini 1871, *Syngnathus tenuirostris* Rathke 1837, *Syngnathus typhle* Linnaeus 1758, *Syngnathus variegatus* Palas 1811. Three of the European syngnathids occur only in the W. Atlantic coastline (*E. aequoraeus, N. lumbriciformis, S. rostellatus*), two in the Black and Azov Seas (*S. schmidti, S. variegatus*), while the rest of the species are also present in the Mediterranean Sea. The two seahorse species (*H. hippocampus* and *H. guttulatus*) are listed in Appendix II of the Bern Convention, while many of the rest of the species are listed in the Annex II of the Directive 92/43/EE in a local level. Most of the syngnathids are listed as "Data Deficient"' on the IUCN Red List, while seahorses as "Vulnerable" or "Endangered".

Research teams all over Europe are working on the biology and phylogenetic relationships of syngnathids. Ambient water temperature, latitude and type of vegetation are considered the main factors influencing their biology. In fact, the above factors have been found to cause major deviations in the intensity of sexual selection, population structure, growth, mating system and reproductive biology between northern and southern populations (e.g. Vincent et al. 1994; Franzoi et al. 1993; Silva et al. 2009, 2010; Malavasi

et al. 2007; Rispoli and Wilson 2008). Phylogeographic studies have explored the population structure of European pipefish and seahorses throughout their distributional range (European Coastline from North to the Black Sea) (Hablutzel 2009; Woodall et al. 2011; Wilson and Veraguth 2010; Sanna et al. 2013a). Three major breaks were found, which are in accordance with the biogeography of the European coastline: a) the Gulf of Biscay separates northern and Atlantic coast populations, b) the Gates of Gibraltar separate Atlantic and Mediterranean populations and c) the Straits of Sicily and Adriatic Sea divide Mediterranean Sea to western and eastern groups (Wilson and Veraguth 2010; Woodal et al 2011). At the same time, morphometric studies show a high plasticity of body shape among continuously distributed inshore populations (Hablutzel 2009; Sanna et al 2013a).

However, most of the existing studies focus on the northern and Atlantic coast populations (e.g. Berglund 1986; Vincent et al. 1994, 1995; Silva et al. 2006b; Sundin 2013; Hubner et al. 2013) and less on the Mediterranean (e.g. Franzoi et al. 1993; Riccato et al. 2003; Alaya et al. 2011; Sanna et al. 2013a).

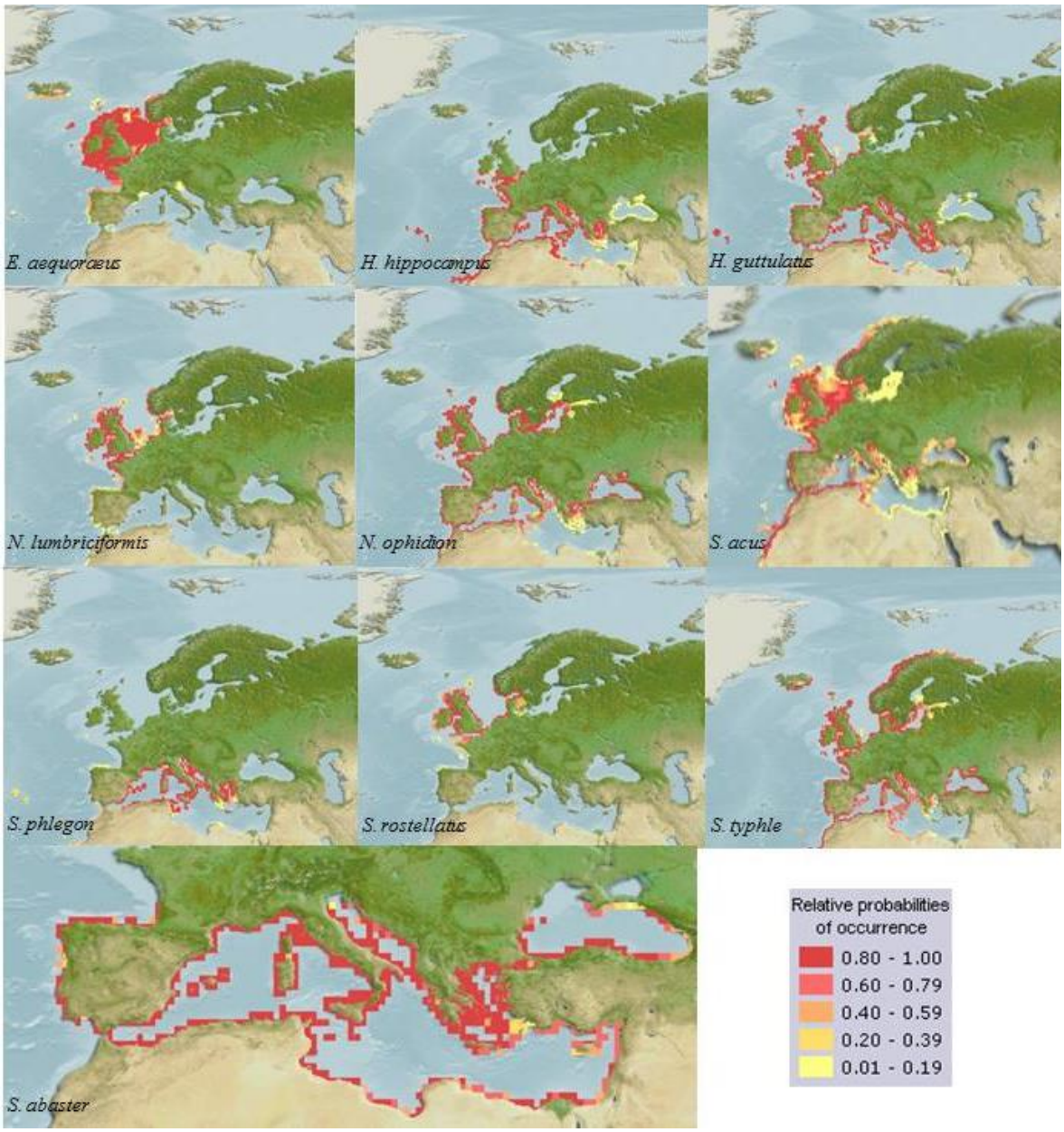

Figure 1.4. Distribution maps of the most abundant European species of the family of Syngnathidae showing the relative probabilities of the species occurrence along the European coastline (E., genus *Entelurus;* H., genus *Hippocampus*; N., genus *Nerophis*; S., genus *Syngnathus*) (modified after www.fishbase.org).

Εικόνα 1.4. Χάρτες κατανομών των πιο διαδομένων ειδών της οικογένειας των Συγναθιδών στους οποίους απεικονίζεται η πιθανότητα εμφάνισης κάθε είδους κατά μήκος της Ευρωπαϊκής ακτογραμμής (E., γένος *Entelurus;* H., γένος *Hippocampus* ; N., γένος *Nerophis*; S., γένος *Syngnathus*) (τροποποιημένος από www.fishbase.org)

1.4. Syngnathids along the coastline of Greece

1.4.1. Syngnathid species

Due to the high degree of vegetation along the Greek coastline nine syngnathid species occur sympatrically: *Hippocampus hippocampus*, *Hippocampus guttulatus*, *Nerophis ophidion*, *Syngnathus abaster*, *Syngnathus acus*, *Syngnathus phlegon*, *Syngnathus typhle*, *Syngnathus taenionutus*, and *Syngnathus tenuirostris*. The first seven were described by Dawson (1986). Papakonstantinou (1988) recorded *S. taenionutus* whereas Economidis and Bauchot (1976) recorded *S. tenuirostris* species.

Studies on the biology of these species in the Ionian and the Aegean Sea are rare (Gurkan and Taskavak 2007; Kitsos et al. 2008; Gurkan et al. 2009, Ράμφος και συν. 2013), while most references are results of bycatch (Koutrakis et al. 2000; Koutrakis and Tsikliras 2003; Liousia et al. 2012; Λεγάκις και Μαραγκού 2009). Also, despite the fact that the existing phylogenetic studies tried to cover the species full distributional range, sampling regions are lacking in the Eastern Mediterranean part. More specifically, they have sampled populations from: i) only one region in the Aegean Sea and none in the Ionian (Woodall et al. 2011) or ii) none at all, with their sampling range stopping at the Adriatic Sea and continuing at the Sea of Marmara (Wilson and Veraguth 2010; Sanna et al. 2013a).

1.4.2. Greek coastline and paleogeography

Greece is situated in the E. Mediterranean region in the Balkan Peninsula. It is surrounded by the Ionian and Aegean Seas. The coastline is around 15.000 km long (Coastal Guide Country File Greece 2001) and exhibits a wide variety of ecosystems between and within the Seas (e.g. from rocky to muddy substratum, vegetated to unvegetated, oligotrophic to eutrophic basins etc.) (Stergiou et al. 1997; Coll et al. 2010).

As already mentioned syngnathid species are highly associated to vegetation (Teixeira and Vieira 1995; Vincent et al. 1995; Erzini et al. 2002). Seagrass occupies extensive areas of the Greek coastline (Lipkin et al. 2003). The little Neptune grass *Cymodocea nodoca* Ascherson 1870 (Figure 1.5a) appears both in shallow and deeper waters mostly at sheltered and to a less extent at exposed to winds and waves beaches. It is found both on sandy and muddy bottoms (Lipkin et al. 2003). The Neptune grass or Mediterranean tapeweed *Posidonia oceanica* (L.) Delile (Figure 1.5b) is found both in shallow and deeper waters on sandy bottoms (up to 35-40 m) (Lipkin et al. 2003). It is an endemic species to the Mediterranean Sea protected by the Habitat Directive 92/43/EU (Annex I), included in the reference list of priority habitats of the SPA/BIO Protocol of Barcelona Convention and in the Water Framework Directive 2000/60/EU as a biological indicator of the ecological status of the Mediterranean marine ecosystems.

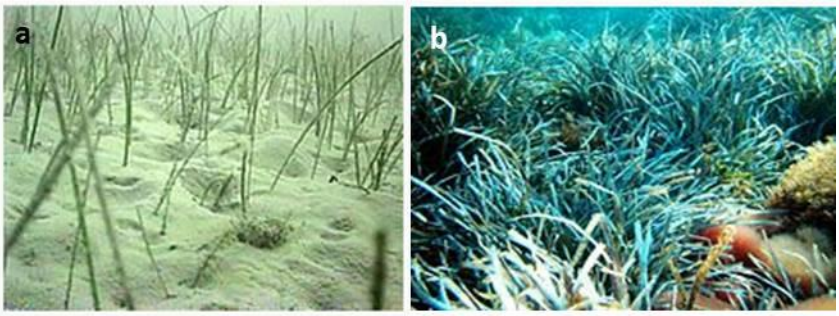

Figure 1.5. Types of seagrass associated with the presence of Syngnathid species along the Greek coastline a) *Cymodocea nodoca* b) *Posidonia oceanica* (after www.wikipedia.og).
Εικόνα 1.5. Τύποι υδρόβιας βλάστησης κατά μήκος της ελληνικής ακτογραμμής που συνδέονται με τη παρουσία μελών της οικογένειας των Συγναθιδών a) *Cymodocea nodoca* b) *Posidonia oceanica* (από www.wikipedia.org)

The Greek Sea is composed of two major biogeographical zones -Ionian and Aegean Sea- which were mostly formed during the Pleistocene events and the subsequent reconnection of Mediterranean Sea to the Marmara and Black Sea (Bianchi et al. 2012).

Particularly, during the Pleistocene period, the levels of the Ionian Sea retreated (though to a moderate extent in comparison to the Aegean, Perissoratis and Conispoliatis 2003). Most islands were connected with each other and with the mainland, forming lakes. The coastline of W. Greece was up to 10 km offshore, while the coast of W. Peloponesus 5-8 km (Perissoratis and Conispoliatis 2003). Also, Korinthiakos and Amvrakikos Gulf formed two lakes (Papatheodorou et al. 1993; Perissoratis et al. 2000; Perissoratis and Conispoliatis 2003).

The palaeomorphology of the Aegean Sea was formed during the Middle-Upper Pleistocene period (Lykousis 2009). During that period, the sea surface was restricted, and more than half of the present Sea was exposed to subaerial conditions (Lykousis 2009). In the northern and central sectors, extended plains and small lakes were formed, while the islands of Thasos and Samothrace formed high mountain ranges (Perissoratis and Van Andel 1991; Lykousis et al. 1995; Perissoratis and Conispoliatis 2003; Lykousis 2009). At the same time, the riverine systems of the N. Balkan and E. Anatolia were discharged to the North Aegean, while the Dardanelles were cut off (Stanley and Blanpied 1980, Lykousis 2009). On the other hand, the Southern Aegean remained more unaffected, because of the steep coastal cliffs (Perissoratis and Conispoliatis 2003).

When the sea levels started to rise again, only few Ionian Sea islands remained connected with the mainland, whereas the Amvrakikos and Korinthiakos Gulfs were lakes (Perissoratis and Conispoliatis 2003). In the Aegean Sea, part of the north sector was still subaerially exposed and Samothrace and Thasos islands were connected (to a smaller extent) with the mainland (Perissoratis and Conispoliatis 2003). Most of the lakes were

overwhelmed by the sea except from the Evoikos Gulf, due to its high entrance barrier (Perissoratis and Van Andel 1991; Perissoratis and Conispoliatis 2003). Most islands, at that time, obtained their present shape (Perissoratis and Conispoliatis 2003). The main source of the water surface circulation was an inflow from the Black Sea (Aksu et al. 1995). In the late Holocene, sea waters flooded all lakes and gulfs and in some cases reached even further than they are observed today. The gradual inflow of the Atlantic Ocean waters eventually outbalanced the outflow of the Black Sea waters around 6400 years ago and led to the contemporary water temperature and salinity levels (Aksu et al. 1995).

1.5. Aim of the thesis

Due to their geographical and temporal diversity, syngnathid species provide an excellent system for comparative evolutionary studies (Mobley and Jones 2009; Mobley et al. 2011; Wilson and Orr 2011). Despite the fact that studies on the biology, mating system and phylogenetic relationships of European syngnathids have tried to include as many species as possible in their whole distributional range, the population of the Ionian and Aegean Seas remains a "black box". This is a major gap in the overview of the species biology and evolution as the Ionian and Aegean Seas: a) consist of a wide range of ecosystems regarding biotic, abiotic, physicochemical and other environmental factors (Stergiou et al. 1997, Coll et al. 2010), b) constitute two major biogeographical zones and c) are important ecosystems of the E. Mediterranean Sea as they are in close proximity to the Marmara, Black and Adriatic Seas.

As already mentioned syngnathids persisted the last major vicariant event, the Pleistocene glaciation, by taking refugium in protected areas and then recolonizing old and new habitats (Lourie and Vincent 2004; Wilson and Veraguth 2010; Alaya et al. 2011; Sanna et al. 2013a; Woodall et al. 2011). The climate changes that occurred during the Pleistocene- and in most cases during the Last Glacial Maximum (LGM) - had an impact on the geographic distribution and genetic structure of many syngnathids (Mobley and Jones 2007; Wilson and Veraguth 2010; Woodal et al. 2011; Sanna et al 2013a).

Most of the existing studies of syngnathids focus on specific isolated populations at different biogeographical zones and therefore provide data only on large scale differences (European or Mediterranean level) (Franzoi et al. 1993; Riccato et al. 2003; Rispoli and Wilson 2008; Wilson and Veraguth 2010; Sanna et al. 2013a). However, information on the biological traits, the mating system and the genetic structure among populations from the same biogeographical zone are rare (Mobley and Jones 2007; Alaya et al. 2011; Mwale 2013). However, local adaptations can play an important role in the evolution of the species as contrary to the drive for panmixia, they occur between geographically close populations living room for substantial differentiation (Kawecki and Ebert 2004; Blanquart et al. 2013; Richardson et al. 2014).

Taking these factors into consideration, the aim of the present doctoral study was to examine the biology, the genetic and phenotypic structure, and the mating system of the sympatrically occurring syngnathids along the Greek coastline. The two most abundant species along the Greek coastline – *Syngnathus abaster* and *Syngnathus typhle*- were used as model organisms.

The biology of the two species was assessed by the examination of the abundance, population structure, sex ratio, operational sex ratio, growth, length-weight relationships and gonadosomatic and hepatosomatic index in two ecosystems of the N. Ionian Sea (Chapter 2). These habitats vary in abiotic factors (type and coverage of habitat, exposure to the open sea etc.) and are almost 80 km apart. Given that i) the abundance and

population structure of syngnathids were found to vary among different ecosystems (Kendrick and Hyndes 2005; Malavasi et al. 2007) and ii) *S. abaster* and *S. typhle* posse different ecological niches (for details see Chapter 2 in the present thesis), it was expected that the above mentioned biological features would differ between i) the specimens of the two examined stations (intra- species level) and ii) the two species (inter-species level).

Genetic structure was examined using the mitochondrial DNA control region and the nuclear locus A1 in specimens of the two species along the coastline of the mainland Greece (Chapter 3). Morphological differences were investigated with the use of a landmark based morphometric protocol and meristic characters (Chapter 4). If local adaptations existed, differences in the genetic structure and the morphological pattern of the two species at least at the level of the two major biogeographical zones (Ionian and Aegean Seas) were expected to be found.

Also, the genetic structure of the Greek samples of *S. typhle* was compared to the existing data (sequences downloaded from Genebank, Benson et al. 2011) of European populations along the species range (Wilson and Veraguth 2010) (Chapter 3). More specifically, given that existing data support isolation by distance dispersal pattern, it was tested whether the specimens from the Ionian and the Aegean Sea cluster with the Western Mediterranean or Marmara/Black Sea group or form a clade of their own. Given that the biogeographical zones of the Ionian and Aegean Sea are distinct from the Adriatic and the Marmara/ Black Sea (Bianchi et al. 2012), it was expected that populations from the Greek coastline would form a clade of their own as revealed by other marine species too (Bahri-Sfar et al. 2000; Magoulas et al. 2006; Sala-Bozano et al. 2009; Yebra et al. 2011).

Finally, both *Syngnathus abaster* and *Syngnathus typhle* species are known to be polygynandrous (Jones et al. 1999; Rispoli and Wilson 2008; Hubner et al. 2013). However, the mating patterns and the intensity of sexual selection may vary between syngnathid populations due to demographic (Mobley and Jones 2007; Rispoli and Wilson 2008) and environmental factors (Rispoli and Wilson 2008; Mobley and Jones 2007, 2009; Wilson 2009; Monteiro and Lyons 2012). Moreover, Rispoli and Wilson (2008) and Mobley and Jones (2009) revealed a correlation between sexual selection and multiple mating of syngnathids isolated populations. Taking these factors into consideration the final goal of the present thesis was to describe the mating system of the two in question pipefish species from the N. Ionian Sea and examine if geographical isolation impacts on the degree of both species polygamy (Chapter 5). If indeed there was an effect, difference in the level of polygamy between the Greek and the rest of the European populations were expected to be found.

Chapter 2. Biological features of *Syngnathus abaster* and *Syngnathus typhle* species in the N. Ionian Sea

2.1. INTRODUCTION

The biology of *Syngnathus abaster* and *Syngnathus typhle* species has been extensively studied over the last decades (e.g. Berglund et al. 1986; Franzoi et al 1993; Vincent et al. 1994; 1995; Silva et al. 2006b).

Syngnathus abaster Risso, 1827 (common name: black-striped pipefish) (Figure 2.1, Table 2.1) is a benthic euryhaline syngnathid species that inhabits freshwater, brackish and marine coastal environments in depths up to 5 m and a temperature range of 8–24 ^{o}C (Dawson 1986; Cakić et al. 2002, www.fishbase.org). As in most pipefish, it is connected with sandy or muddy bottoms, in seagrass meadows or macroalgal beds, such as *Cymodocea nodoca*, *Posidonia oceanica and Zostera marina* (Dawson 1986; Franzoi et al. 1993; Silva et al. 2006b). The species color varies from light green to dark brown depending on the characteristics of the environment that it inhabits (Dawson 1986). Its distribution range includes the Mediterranean and Black Sea, as well as the European Atlantic Coast, southern to the Biscay Gulf (Dawson 1986) (Figure 2.2). The bony plates around the species body constitute it a slow swimmer, limiting, thus, its potential for dispersal.

Figure 2.1 Female individuals of *S. abaster* species (common name: black-striped pipefish) (after R. Pillon).
Εικόνα 2.1. Θηλυκό άτομο του είδους *S. abaster* (κοινή ονομασία: σακκοράφα, ταινιοσακκοράφα, κατουρλίδα) (από R. Pillon).

Table 2.1. Systematic classification of *S. abaster* species
Πίνακας 2.1. Συστηματική κατάταξη του είδους *S. abaster*.

Kingdom:	Animalia
Phylum:	Chordata
Class:	Actinopterygii
Order:	Syngnathiformes
Family:	Syngnathidae
Subfamily:	Syngnathinae
Genus:	*Syngnathus*
Species:	***S. abaster***

The studies of Franzoi et al. (1993), Riccato et al. (2003) and Silva et al. (2006b, 2008, 2010) shed light on the species biology. According to them, *S. abaster* is characterized by a short life cycle (around 18 months). The species reaches sexual maturity within the first three to four months, corresponding to a total length of about 6 cm. The mean observed length is 8.5-12.5 cm (Franzoi et al. 1993; Riccato et al. 2003), while the maximum recorded length is 21 cm (www.fishbase.org). Adult specimens are slow moving suction feeders whose diet remains the same throughout their lifespan (Franzoi et al. 1993). Juvenile specimens prey on phytal organisms (Franzoi et al. 1993). Sex ratio is unbiased, in the so far studied populations (Franzoi et al 1993; Riccato et al. 2003; Cunha 2012).

The reproductive period is well defined lasting for almost six months (approximately April- October) while exact duration depends on ambient temperature (Franzoi et al. 1993; Riccato et al. 2003; Silva et al. 2010). During the reproductive season, pregnant males brood their embryos in a closed marsupium located on the tail (Urophori). (Herald 1959; Carcupino 1997; Wilson 2001; Silva et al. 2006a; Cuhna 2012). The female-female competition, the moderate levels of sexual size dimorphism and the female-biased operational sex ratio indicated that the species is sex role reversed (e.g. Silva et al. 2006b).

The high phenotypic variability of the species has misled many researchers into classifying different populations as sub-species or even worse as new species (www.fishbase.org). However, nowadays almost all of them are synonymized and considered the same species.

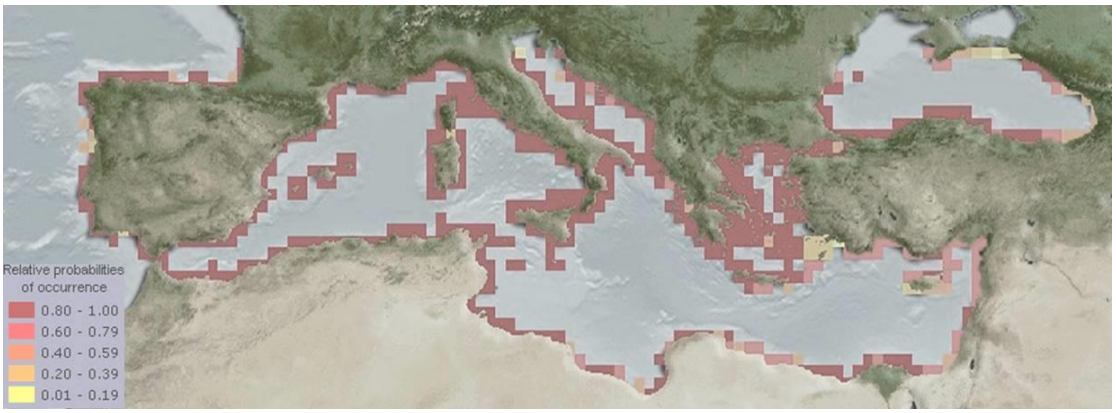

Figure 2.2. Distribution map of *S. abaster* species showing the relative probabilities of the species occurrence (modified after www.fishbase.org).
Εικόνα 2.2. Χάρτης κατανομής του είδους *S. abaster* στον οποίο απεικονίζεται η πιθανότητα εμφάνισης του είδους (τροποποιημένος από www.fishbase.org).

Syngnathus typhle Linnaeus, 1758 (common name: broadnosed pipefish) (Figure 2.3, Table 2.2) is a marine species which prefers shallow waters (depths from 0.6 to 6 m) (Dawson 1986, www.fishbase.org). It is connected with sandy or muddy bottoms, covered with phytobentos such as *Cymodocea nodoca*, *Posidonia oceanica and Zostera marina Zostera marina*), where it mostly swims vertically but also rests (Vincent et al. 1995; Skóra 2001, according to Tarnowska and Sapota 2007; Malavasi et al. 2007). The species color ranges from light green to nearly black and the coloration pattern from uniformly colored with almost no contrast to highly contrasted dark stripes. The different colors closely match the varying colors of the eelgrass (Scliwa 1986 according to Bernet et al. 1998). It is found along the European Atlantic coasts from mid-Norway to southwards in Morocco as well as in the Mediterranean Sea, the Black Sea and the Azov Sea (Figure 2.4) (Dawson 1986). The bony plates around the species body constitute it a slow swimmer, limiting, thus, its potential for long distance migration.

Figure 2.3 Male individual of *S. typhle* species (common name: broadnosed pipefish) (after R. Pillon).
Εικόνα 2.3. Θηλυκό άτομο του είδους *S. typhle* (κοινή ονομασία: κατουρλίδα) (από R. Pillon).

Table 2.2. Systematic classification of *S. typhle* species
Πίνακας 2.2. Συστηματική κατάταξη του είδους *S. typhle.*

Kingdom:	Animalia
Phylum:	Chordata
Class:	Actinopterygii
Order:	Syngnathiformes
Family:	Syngnathidae
Subfamily:	Syngnathinae
Genus:	*Syngnathus*
Species:	***S. typhle***

S. typhle specimens have a life span of 2-3 years, reaching sexual maturity at about 11 cm. The mean observed length is 15 cm (Oliveira et al. 2007) and the maximum 35 cm (Muss and Nielsen 1999; www. fishbase.org). Adult specimens are slow moving suction feeders with a progressive dietary shift towards larger and fast moving preys (Oliveira et al. 2007). Sex ratio is usually unbiased (Berglund et al. 1986; Vincent et al. 1995; Franzoi et al. 1993). During cold winter months individuals migrate to deeper and more protected waters (Vincent et al. 1995; Tarnowska and Sapota 2007).

Most studies on *S. typhle* focus mainly on its reproductive biology and ethology (e.g. Berglund 1993; Ahnesjo 1996; Rispoli and Wilson 2008). These studies showed that the species has a well-defined reproductive period during spring, summer and autumn months. The exact duration depends on ambient temperature (e.g. Berglund et al. 1986; Riccato et al. 2003; Sundin 2013). The species belong to the group of Urophori with a closed brood pouch (Herald 1959; Carcupino 1997; Wilson 2001). Female- female competition, moderate levels of sexual size dimorphism and female biased operational sex ratio (OSR) indicate that the species is sex role reversed (e.g. Berglund et al 1986; Clutton-Brock and Vincent 1991; Vincent et al. 1994).

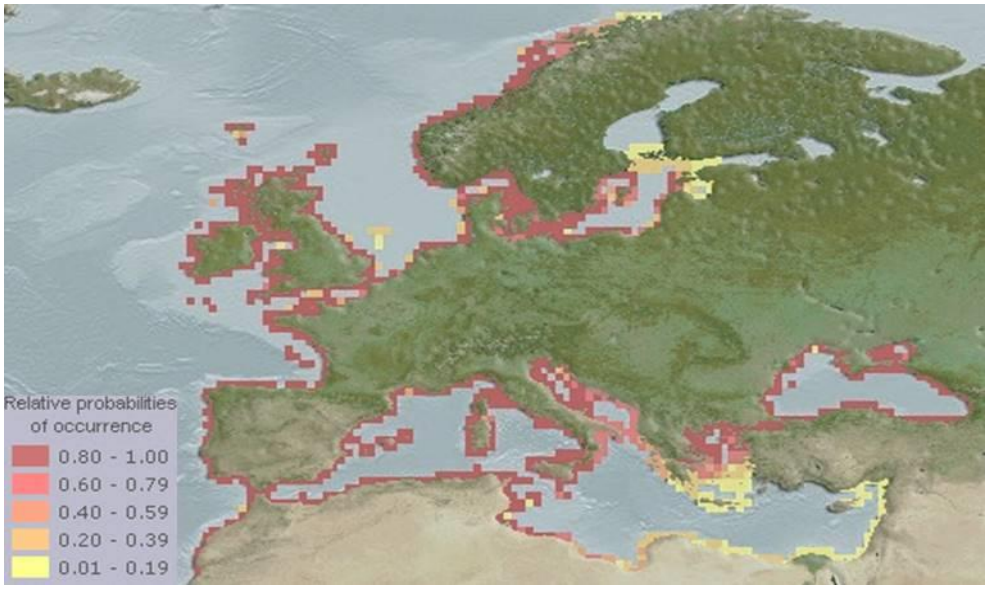

Figure 2.4. Distribution map of *S. typhle* species showing the relative probabilities of the species occurrence (modified after www.fishbase.org).
Εικόνα 2.4. Χάρτης κατανομής του είδους *S. typhle*, στον οποίο απεικονίζεται η πιθανότητα εμφάνισης του είδους (τροποποιημένος από www.fishbase.org).

Even though, the two species occur sympatrically, they tend to diverge in terms of microhabitat use, exhibiting a strong habitat partitioning; *S. typhle* specimens occupy the high and the intermediate portion of the canopy, while *S. abaster* are benthic (Malavasi et al. 2007). Moreover, *S. typhle* mostly occurs in *Cymodocea* seagrass, while *S. abaster* in eelgrass (*Zostera marina*) (Malavasi et al. 2007). These patterns verify the hypothesis of generic spread i.e. congeneric species avoid competition by partitioning their habitats (Tokeshi 1999) and have been observed in other syngnathid species too (Steffe et al. 1989; Curtis and Vincent 2006; Kendrick and Hyndes 2005).

However, these studies provide information mostly on species abundance. There is limited information on how different habitats can affect the species biological cycle. As already mentioned, both *S. abaster* and *S. typhle* have a wide distributional range that include E. Atlantic, Mediterranean and Black Sea coasts. The biotic (species structure) and abiotic (e.g. temperature, salinity, turbidity) factors of these habitats vary (Coll et al. 2010; Bianchi et al. 2012). The only common factor among these varying habitats for the two species in question is vegetation. However the type and the density of vegetation among such range also differs (Sales et al. 2012).

Existing studies on other species- with such wide distribution- have shown a strong correlation between biological cycles and different habitats (Brander 1995; Portner et al 2001; Tsuzuki et al. 2007). Temperature, salinity and trophic levels are considered among the most important abiotic factors that influences biology (e.g. Brander 1995; Portner et al. 2001; Lappalainen et al. 2001; Stergiou 2000). The Greek coastline is hypervariable exhibiting a wide range of trophic levels (mostly oligotrophic but highly eutrophic basins are not an exception), physicochemical (temperature, salinity etc.) and other environmental- abiotic (different types of substratum, and vegetation, vegetation coverage, currents etc.) parameters (Stergiou et al. 1997; Coll et al. 2010). Under these diverse conditions, biological cycles and life history traits of fish stock were found to differ both between Greek and Mediterranean populations but also among Greek localities (Stergiou et al. 1999; 2000; 2007).

So, taking into account: a) the wide distributional range, the vegetation preferences, the habitat partitioning and the influence of temperature on the duration of the reproductive period of *S. abaster and S .typhle* (Franzoi et al. 1993; Riccato et al. 2003; Malavasi et al. 2007), b) the hypervariable Greek coastline (Stergiou et al. 1997; Coll et al. 2010) and c) the strong habitat-related influence in the biology of marine organism (Stergiou et al. 1999; 2000; 2007) an interesting question arises: What are the biological features of *S. abaster* and *S. typhle* species in the N. Ionian Sea?

This question was the main axe of the present study. Population structure, sex ratio, operational sex ratio, growth, size at maturity, length-weight parameters, gonadosomatic and hepatosomatic indices of *S. abaster* and *S. typhle* were explored in two different ecosystems; Drepano (Igoumenitsa Gulf) and Neochori (Amvrakikos Gulf). Drepano station has a sandy substratum which is characterized by patchy seagrass (*Cymodocea*

nodosa) and bare sand, whereas, Neochori muddy substratum has a full coverage of dense vegetation (*Posidonia oceanica*). Given the difference in the type of the species-specific vegetation preference it was expected that Drepano station would be a more suitable environment for *S. typhle* individuals and a less suitable for *S. abaster* compared to Neochori station (Malavasi et al. 2007). This factor led into the hypothesis that the biology of the two studied species could be influenced by the type and coverage of vegetation and could differ between Drepano and Neochori stations.

2.2. MATERIALS AND METHODS

2.2.1. Sampling stations

The sublittoral zone of the N. Ionian Sea was searched in order to find satisfactory sampling stations for our study (i.e. abundance of pipefish, differences in the type and coverage of vegetation and other abiotic conditions). The sampling stations that met these criteria more effectively were Drepano and Neochori (Figure 2.5).

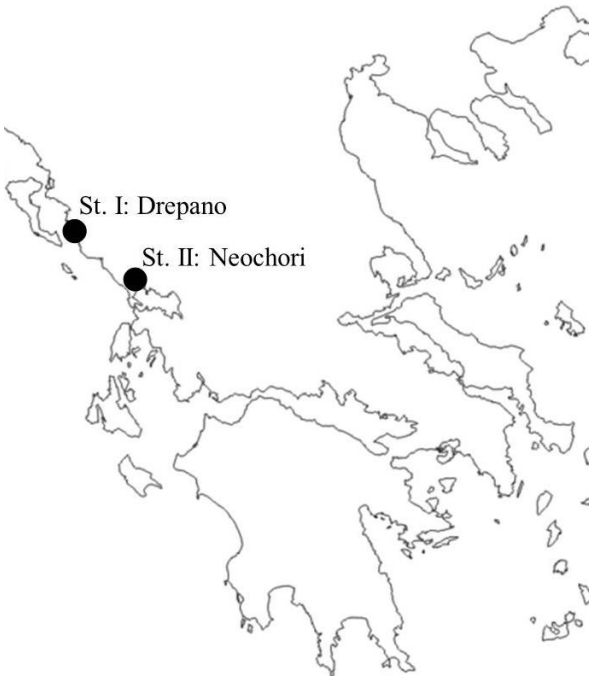

Figure 2.5. Map of the mainland of Greece indicating the two sampling stations of the present study. St. I: Drepano (Gulf of Igoumenitsa 39º31′04.63″N, 20º13′29.38″E), St. II: Neochori (Amvrakikos Gulf 39º00′10.92″N, 20º45′22.35″E).
Εικόνα 2.5. Χάρτης της ηπειρωτικής Ελλάδας στον οποίο απεικονίζονται οι δύο σταθμοί δειγματοληψίας της παρούσας μελέτης. Στ. Ι: Δρέπανο (Κόλπος Ηγουμενίτσας 39º31′04.63″N, 20º13′29.38″E), Στ. ΙΙ: Νεοχώρι (Αμβρακικός Κόλπος 39º00′10.92″N, 20º45′22.35″E)

The station of Drepano (Gulf of Igoumenitsa 39º31′04.63″N, 20º13′29.38″E) (Figure 2.6) is situated in the North part of the Igoumenitsa Gulf. It is in close association with the Kalamas River estuarine ecosystem and is exposed to wind and waves. Its sandy substratum comprises predominantly of *Cymodocea nodosa*, providing a landscape of patchy seagrass amongst areas of bare sand. The water temperature, concentration of dissolved oxygen and salinity ranged from 9 to 28 ^{o}C, 8.14 to 9.90 mg/l and 33.60 to 40.25 psu respectively (Figure 2.7). The concentration of nutrients (NO_2^-, NO_3^-, PO_4^{3-}) and chlorophyll-a also varied in a monthly basis (Figure 2.7). According to the trophic index for marine ecosystems (TRIX) (Vollenweider et al. 1998), Drepano is characterized as an oligotrophic ecosystem (Figure 2.8). The ichthyofauna of the station comprised of 17 families, 23 genera and 37 species (Table 2.1 in Appendix). The species of the Syngnathidae family accounted for the 3.22% of the station's total abundance (Figure 2.9).

Figure 2.6. General aspect of the Drepano and Neochori sampling stations.
Εικόνα 2.6. Γενική άποψη των σταθμών δειγματοληψίας του Δρεπάνου και του Νεοχωρίου.

The station of Neochori (39º00′10.92″N, 20º45′22.35″E) (Figure 2.6) is situated in the North-West part of the Amvrakikos Gulf and is in close affinity with the Mazoma lagoon. It is a sheltered station protected from wind and waves. Its muddy substratum is completely covered by dense vegetation, comprising mainly of *Posidonia oceanica*. The water temperature, concentration of dissolved oxygen and salinity ranged from 11.74 to 32.10 ^{o}C, 8.00 to 12.50 mg/l and 29.20 to 37.90 psu respectively (Figure 2.7). The concentration of nutrients (NO_2^-, NO_3^-, PO_4^{3-}) and chlorophyll-a also varied on a monthly basis (Figure 2.7). Even though Amvrakikos Gulf is considered an eutrophic ecosystem, based on the trophic index for marine ecosystems (TRIX) (Vollenweider et al. 1998), Neochori is characterized as oligotrophic (Figure 2.8). The ichthyofauna of the station comprised of 15 families, 19 genera and 24 species (Table 2.2 in Appendix). The species of the Syngnathidae family accounted for the 11 % of the station's total abundance (Figure 2.9).

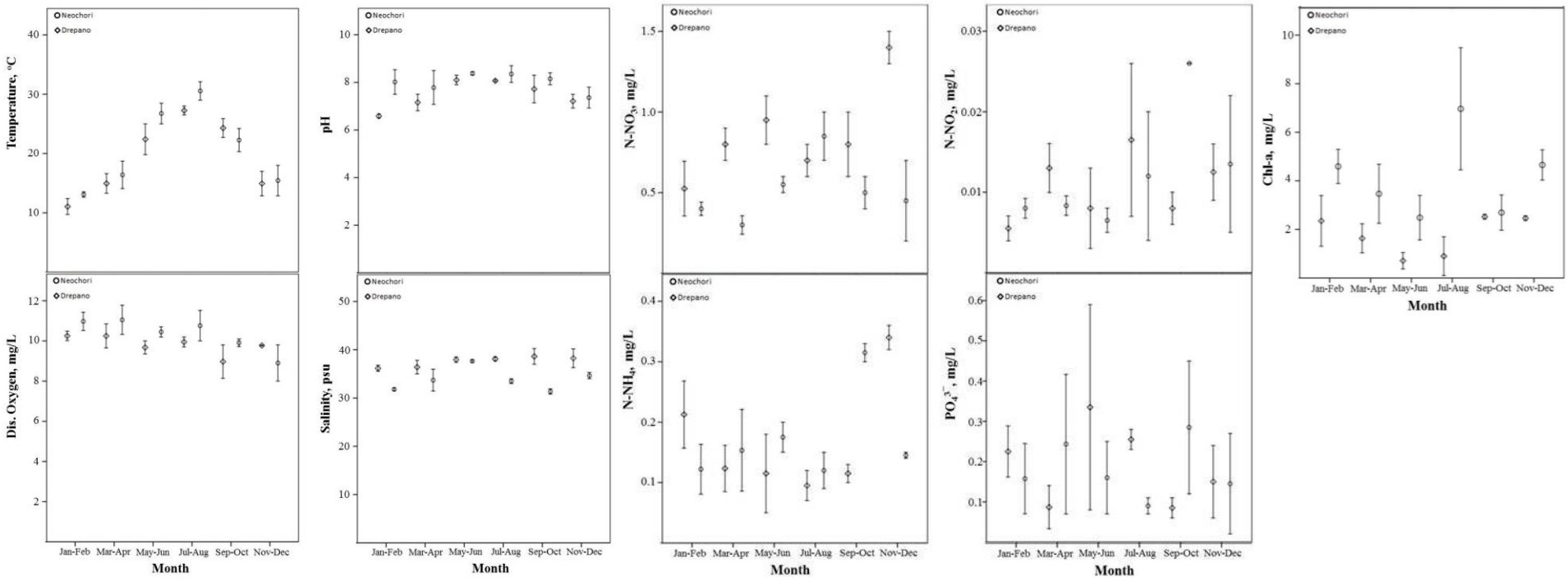

Figure 2.7. Bimonthly variation of water temperature (°C), dissolved oxygen concentration (mg/l), pH values, salinity (psu) and concentration (mg/L) of phosphate (PO_4^{3-}), nitrates ($N\text{-}NO_2$), nitrites ($N\text{-}NO_2$) ammonia ($N\text{-}NH_4$) and chlorophylls (Chl- a) in Drepano and Neochori stations in the present study (the T-bars that extend from the boxes+ correspond to the standard error of the sample).

Εικόνα 2.7. Διμηνιαία διακύμανση της θερμοκρασίας (°C), της συγκέντρωσης του διαλυμένου οξυγόνου (mg/l), των των ιόντων του pH, της αλατότητας (psu) και της συγκέντρωσης (mg/l) των ορθοφωσφωρικών ριζών (PO_4^{3-}), των νιτρωδών ($N\text{-}NO_2$), των νιτρικών ($N\text{-}NO_3$), αμμωνιακών ($N\text{-}NH_4$) και χλωροφυλλών (Chl-a) στου σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της παρούσας μελέτης (οι γραμμές σχήματος «Τ» αντιστοιχούν στο τυπικό σφάλμα του δείγματος).

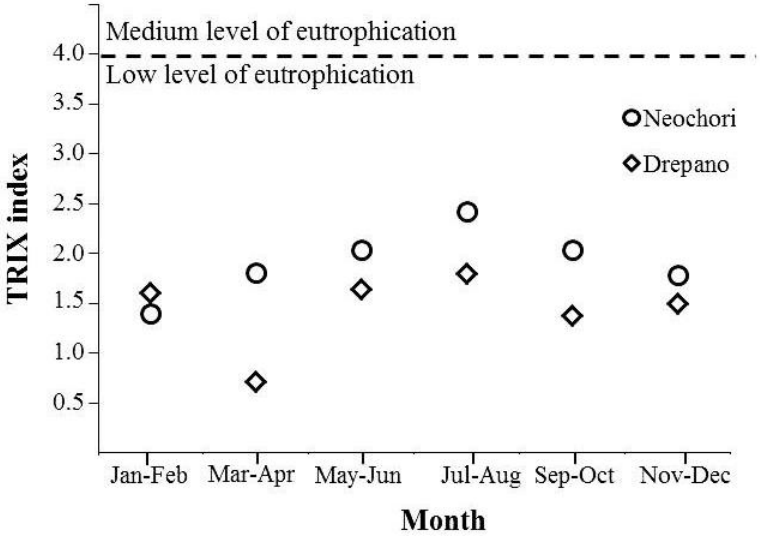

Figure 2.8. Bimonthly variation of the trophic index for marine ecosystems (TRIX) for the sampling stations of Drepano and Neochori in the present study (the dashed line indicates the limit of the low and medium level eutrophic ecosystems).
Εικόνα 2.8. Διμηνιαία μεταβολή του δείκτη ευτροφισμού των θαλάσσιων οικοσυστημάτων (TRIX) για τους σταθμούς δειγματοληψία του Δρεπάνου και του Νεοχωρίου στη διάρκεια της παρούσας μελέτης (η διακεκομμένη γραμμή απεικονίζει το όριο μεταξύ του ολιγότροφου και μεσότροφου οικοσυστήματος).

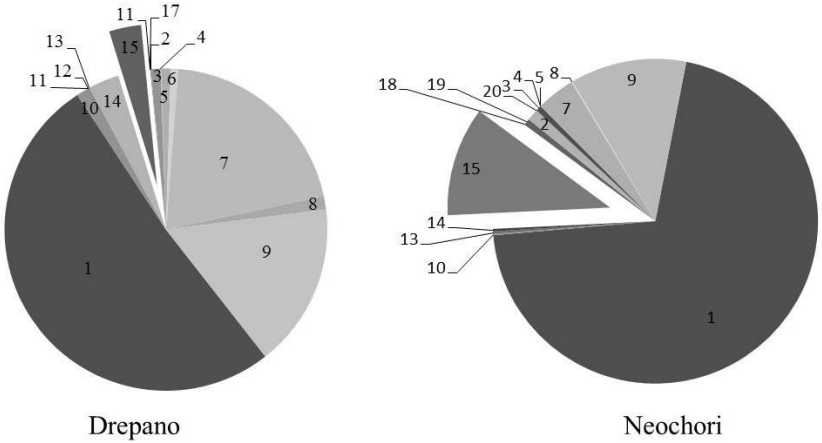

Figure 2.9. Relative abundance (%) of the sampled families from the ichthyofauna of Drepano and Neochori stations in the present study (1.Atherinidae, 2.Belonidae, 3.Blenniidae, 4.Callionymidae, 5.Clupeidae, 6.Engraulidae, 7.Gobidae, 8.Labridae, 9.Mugilidae, 10.Mullidae, 11.Scophthalmidae, 12.Scorpaenidae, 13.Soleidae. 14.Sparidae, 15.Syngnathidae, 16.Trachinidae, 17.Triglidae, 18.Anguillidae, 19.Cyprinodontidae, 20. Sciaenidae).
Εικόνα 2.9. Σχετική αφθονία (%) των οικογενειών της ιχθυοπανίδας των σταθμών του Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της παρούσας μελέτης (1.Atherinidae, 2.Belonidae, 3.Blenniidae, 4.Callionymidae, 5.Clupeidae, 6.Engraulidae, 7.Gobidae, 8.Labridae, 9.Mugilidae, 10.Mullidae, 11.Scophthalmidae, 12.Scorpaenidae, 13.Soleidae. 14.Sparidae, 15.Syngnathidae, 16.Trachinidae, 17.Triglidae, 18.Anguillidae, 19.Cyprinodontidae, 20. Sciaenidae).

2.2.2. Sampling method

Fish species were collected at each station on a monthly basis from January of 2009 to March of 2010, using a beach seine (16 m long and 1.5 m high, mesh size of 2–4 mm, without a bag) (Figure 2.10). Weights on the bottom and buoys on the top kept the net in a firm vertical position while hauling. The nets were attached to 1.5-m long wooden poles on either end, providing a firm grasp while hauling. The seine was set parallel to the shore -in water depth up to 1.4 m- and then pulled back to the beach (Figure 2.10). While hauling, the seine remained in constant contact with the bottom. In each station 4 hauls were attempted, covering a standardized total area of 160 m^2. Between consecutive hauls a 20 minute interval existed in order to reduce habitat disturbance. Existing studies indicate that the beach seine can capture pipefishes in high abundances along the sublittoral zone of marine and brackish waters (Franco et al. 2006; Malavasi et al. 2007).

Additional sampling effort was conducted with a hand net (30 cm high and 40 cm long, mesh size of 2 mm) (Figure 2.10). The same area as in the beach seine effort was searched following a meandrous pattern move. In this way individuals that escaped the seine were swept away and trapped in the net.

Fish were preserved in neutralized formaldehyde solution (4%) for later laboratory analysis. To maintain standard conditions: i) samplings were always conducted within the first days of each month, between 10 am and 2 pm and ii) sampling effort (number of hauls and covered area) remained the same.

Figure 2.10. Sampling efforts with beach seine and hand net as conducted in the present study.
Εικόνα 2.10. Δειγματοληπτικές μέθοδοι με γρίπο και απόχη όπως πραγματοποιήθηκαν στη παρούσα μελέτη

2.2.3. Species identification- Data collection

In the laboratory, each specimen was identified to the species level based on the key for European syngnathids (Dawson 1986). The number of individuals, total length, as well as total and eviscerated (net) weight, were measured to the nearest 0.1 mm and 0.001 gr, respectively. The gonads and the liver were removed and weighed separately to the nearest 0.001 gr. Sex was determined macroscopically (males were characterized by the presence of brood pouch and females of the same size by the absence of the pouch and the swollen ovipositor region) and -when needed- microscopically with body dissection. When the identification was not possible (gonads were empty or not shaped) specimens were classified as "unsexed". Males were considered brooding/pregnant when at least one egg or embryo was present in the brood pouch and non- brooding when the pouch was empty.

2.2.4. Data analysis

Male, female and unsexed individuals of *S. abaster* and *S. typhle* species were collected on a monthly basis in each station. In order to maximize the number of individuals within each month, in both stations preliminary analyses were conducted (correlation tests, Mann-Whitney U test and Kruskal-Wallis ANOVA). The grouping that best fitted the above mentioned prerequisite was the analysis of the samples on a bimonthly basis; January- February (Jan-Feb), March- April (Mar-Apr), May- June (May-Jun), July-August (Jul-Aug), September- October (Sep-Oct), November-December (Nov-Dec). Samples from January 2010, February 2010 and March 2010 were merged with their corresponding months of 2009. Thus, the analyses of the biology of both species were performed on the above described bimonthly basis using SPSS ver. 21 software.

2.2.4.1. Population structure

For each species, the number of individuals and their lengths were compared within and among Drepano and Neochori stations on a bimonthly basis. Since the sampling area in both station remained the same (standardized total area of 160 m^2) throughout the study direct comparison of the relative abundance was possible. Nonparametric tests were used in all analyses, as the assumptions of parametric tests were not met. Comparisons among groups were made by means of Mann-Whitney U test and Kruskal-Wallis ANOVA (Zar 1999). Post hoc and Bonferroni's correction for multiple comparisons were conducted when significant differences were found.

2.2.4.2. Sex ratio

The sex ratios of males to females and adult (mature) to unsexed specimens of *S. abaster* and *S. typhle* species from Drepano and Neochori stations were calculated on a bimonthly basis. Pearson chi-square test (Zar 1999) was used to assess whether sex ratios were different from 1. The significance level was set to 0.05. In order to estimate each population's overall sex ratio of i) males to females and ii) adult to unsexed specimens the G-test was performed (Zar 1999). G test was used instead of the chi-square test as it has an additive effect and is a better depicter of the overall variation (Zar 1999).

2.2.4.3. Operational sex ratio (OSR)

Operational sex ratio (OSR) was estimated as the proportion of sexually active, non-brooding males to sexually active females (Emlen and Oring 1977) of *S. abaster* and *S. typhle* species from the stations of Drepano and Neochori on a bimonthly basis during the breeding season (March- October). Pregnant males were excluded, as suggested by Kvarnemo and Ahnesjo (1996). During the reproductive period, all female individuals

were considered as being ready to mate since they had mature ovaries. Significance level and the evenness of the ratio were tested as described in the sex ratio analysis.

2.2.4.4. Length classes

Length classes were estimated by the Length Frequency Distribution. The method tabulates the length measurements of fish on a frequency basis and interprets the modes in the distribution as age groups-classes of the population or sample used (Bagenal and Tesch 1978). Therefore it provides information on the spatial and temporal presence and size of individuals at particular location and time (Bagenal and Tesch 1978). This is an indirect method for the age estimation of a population.

In order to estimate length classes of the *S. abaster* and *S. typhle* species from Drepano and Neochori stations, bimonthly charts of total length frequency distribution were plotted for male, female and unsexed individuals. The peaks on the histogram corresponded to distinct length groups, implying the corresponding age groups.

2.2.4.5. Size at maturity (L_{50})

L_{50} is the average size of a population when 50% of the specimens are mature (Trippel and Harvey 1991). Since there was an overlap between the largest immature fish and smallest mature fish, the L_{50} of *S. abaster* and *S. typhle* species from the stations of Drepano and Neochori were assessed by logistic regression analysis. Logistic equations are used to estimate L_{50} because these mathematical functions can follow a cumulative normal curve where L_{50} correspond to normal average length. Sex status (mature/immature) was used as dependent variable, total length as covariate and species, sex and stations as factors interacting with total length. The Hosmer and Lemeshow goodness-of-fit test was used to evaluate the fit of the data to the logistic regression model for each species (Hosmer and Lemeshow 2000).

2.2.4.6. Length-weight relationship (LWR)

The relations between the total length (*L*) and weight (*W*) of *S. abaster* and *S. typhle* individuals from the Neochori and Drepano populations was expressed by the equation:

$$W = a*L^b$$

where, *W* the total weight, *L* the total length, *a* the coefficient related to body form and *b* the exponent indicating isometric growth when equal to 3 and allometric growth when

different to 3 (positive if $b > 3$, negative if $b < 3$) (Beverton and Holt 1957; Froese 2006). The parameters *a* and *b* of the length–weight relations were estimated by the least-square method based on the predictive or Type I linear regression model (Sokal and Rohlf 1995), using *"log W"* as the dependent variable and "log *L"* as the independent variable,

$$\log (W) = \log (a) + b \log (L).$$

Analysis of covariance (ANCOVA) (Sokal and Rohlf 1995) was used to determine whether or not there were significant differences in length–weight relationships between sexes, stations and months during the reproductive period (Le Cren 1951) separately for the two species. Log(W) was used as dependent variable, log(L) as covariate and sampling station, sex and periods (bimonths) as factors. A significant main effect of a factor means that this factor influences the condition of fish in all lengths (the intercept of the LWR-line is shifted), whereas a significant interaction of a factor with the covariate means that both the coefficients *a* and *b* of the LWR are affected (Sokal and Rohlf 1995; Zar 1999).

2.2.4.7. Gonadosomatic Index (GSI)

The gonadosomatic index (GSI) (Yuen 1955) is the ratio of fish gonad weight to body weight and is determined by the following formula

$$GSI = 100*GW/BW$$

where, GW is the gonad weight (g) and BW is the body weight (total or net) (g).

GSI is often used as an indicator of the reproductive activity as the gonads of both males and females increase in size before reproduction and shrink at the end of the reproductive period (e.g. Rajasilta et al. 1997; Ceballos-Vázquez and Elorduy-Garay 1998; Arellano-Martínez and Ceballos-Vázquez 2001; Sadekarpawar and Parikh 2013).

In the present study GSI was estimated on a bimonthly basis for male and female individuals of *S. abaster* and *S. typhle* species from the stations of Drepano and Neochori, with respect to net body weight. In pregnant males embryos and eggs were also removed from the pouch. Ovaries of gravid females increased in size just prior to spawning. Therefore, the peak of the GSI values indicated the beginning of the reproductive season. Also, the GSI of males increases prior the acceptance of a brood, drop off during brooding, and increase again just prior to the release of a brood and acceptance of a new batch of eggs (Mayer 1993, Kornienko 2001). The peak and fall of GSI values indicated the start and the end of the reproductive period. Analysis of covariance (ANCOVA) (Sokal and Rohlf 1995) was used determine whether or not there were significant differences in GSI values between sexes, stations and months during the reproductive period separately for the two species (Le Cren 1951). Weight was used as dependent variable, length as covariate and sampling station, sex and month as factors.

2.2.4.8. Hepatosomatic Index (HSI)

The hepatosomatic index (HSI) (Wootton et al. 1978) is the ratio of fish liver weight to body weight and is determined by the following formula:

$$HSI = 100*LW/BW$$

where, LW is the liver weight (g) and BW is the body weight (total or net) (g).

HSI is used as an estimator of the energy status of the fish; since the liver is an important store of energy (Wootton et al. 1978; Campbell and Love 1978). It was estimated on a bimonthly basis for male and female individuals of *S. abaster* and *S. typhle* species from the station of Drepano and Neochori, with respect to net body weight. In pregnant males embryos and eggs were also removed from the pouch. HSI statistical analysis was the same as described for the GSI.

2.3. RESULTS

2.3.1. *Syngnathus abaster species*

2.3.1.1. Population structure

A total of 578 specimens of *S. abaster* species were caught in Drepano (n=313) and Neochori (n=265) stations during the present study. The relative abundance of sampled specimens was not statistical different between the two stations (Mann- Whitney test: U= 116.500, p> 0.05), whereas the number of caught individuals between the periods differed in each station (Kruskal- Wallis test for Drepano station: H= 13.501, p<0.05; Kruskal-Wallis test for Neochori station: H= 13.524, p<0.05). In particular the highest relative abundance in Drepano station was recorded in July-August period while the lowest during the winter period (Figure 2.11).At the same time, the relative highest abundance in Neochori station was recorded in September-October period while the lowest during the winter period and early spring (Figure 2.11). However, between the two stations the only statistical significant difference in the abundance was found in the November-December period when the number of specimens in Neochori station was marginally higher than in Drepano (Kruskal- Wallis test: H= 3.887, p=0.05).

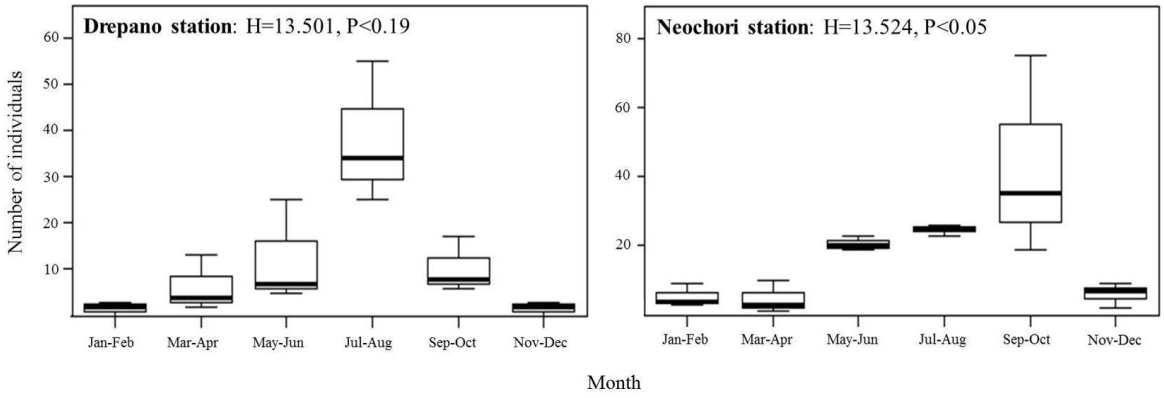

Figure 2.11. Box plot of the average number of individuals of *S. abaster* species caught bimonthly in Drepano and Neochori stations in the present study (*H*, Value of Kruskal-Wallis non parametric test; *P*, level of significance, the middle bold black line within each box corresponds to the median and the T-bars that extend from the boxes correspond to the standard deviation).

Εικόνα 2.11.Θηκόγραμμα του μ.ο. του αριθμού των ατόμων του είδους *S. abaster* τα οποία συλλεχθήκαν ανά δίμηνο στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της παρούσας μελέτης (*H*, η τιμή του μη- παραμετρικού τεστ Kruskal-Wallis; *P*, επίπεδο σημαντικότητας, η μεσαία έντονη γραμμή κάθε θηκογράμματος αντιστοιχεί στο διάμεσο, και οι γραμμές σχήματος «Τ» στην τυπική απόκλιση του δείγματος)**.**

Table 2.3. Mean total length (TL mm) of male, female, unsexed and sexes combined individuals of *S. abaster* species caught in Drepano and Neochori station in the present study (*N*, number of individuals; *MTL*, mean total length*; Min*, minimum total length; *Max*, maximum total length*; St.Dev*, standard deviation of total length)

Πίνακας 2.3. Μέσο ολικό μήκος (TL mm) των αρσενικών, θηλυκών, αδιευκρίνιστου φύλου και για το σύνολο των ατόμων του είδους *S. abaster* τα οποία συλλέχθηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά η διάρκεια της παρούσας μελέτης (*N*, αριθμός ατόμων; *MTL*, μέσο ολικό μήκος*; Min*, ελάχιστο ολικό μήκος; *Max*, μέγιστο ολικό μήκος*; St.Dev*, τυπική απόκλιση ολικού μήκους).

		Males					Females					Unsexed					Total				
Station	**Month**	**N**	**MTL**	**Min**	**Max**	**St.Dev**	**N**	**MTL**	**Min**	**Max**	**St.Dev**	**N**	**MTL**	**Min**	**Max**	**St.Dev**	**N**	**MTL**	**Min**	**Max**	**St.Dev**
	Jan-Feb	9	96.8	75.0	113.0	1.16	4	90.8	80.0	103.0	1.02	3	62.0	58.0	65.0	0.36	16	88.8	58.0	113.0	1.63
	Mar-Apr	3	141.3	96.0	222.0	6.82	10	108.4	77.0	253.0	5.08	1	28.0	28.0	28.0		14	109.7	28.0	253.0	5.68
	May-Jun	19	97.7	82.0	121.0	1.3	23	98.1	78.2	125.1	1.25	20	58.9	34.0	78.0	1.18	62	85.3	34.0	125.1	2.16
Drepano	Jul-Aug	23	94.5	75.0	117.0	1.06	25	107.4	81.0	230.0	3.23	26	61.3	42.0	77.0	0.86	74	87.2	42.0	230. 0	2.79
	Sep-Oct	35	118.6	81.0	214.0	4.29	75	103.3	78.0	220.0	3.12	19	68.0	52.0	76.0	0.8	129	102.3	52.0	220.0	3.60
	Nov-Dec	7	140.7	90.0	211.0	5	9	114.7	80.0	219.0	3.99	2	61.5	47.0	76.0	1.91	18	118.9	47.0	219.0	4.70
	Total	96	109.0	75.0	222.0	3.55	146	103.9	77.0	253.0	3.14	71	62.0	28.0	78.0	1.10	313	95.9	28.0	253.0	3.48
	Jan-Feb	2	102.0	95.0	109.0	0.85	3	115.0	105.0	134.0	1.59	0					5	109.8	95.0	134.0	1.39
	Mar-Apr	2	98.5	77.0	120.0	2.97	4	75.3	70.0	78.0	0.33	13	57.5	44.0	69.0	0.79	19	65.6	44.0	120.0	1.63
	May-Jun	7	86.1	76.0	95.0	0.77	5	95.2	80.0	134.0	2.2	25	49.7	23.0	73.0	1.18	37	62.7	23.0	134.0	2.25
Neochori	Jul-Aug	25	95.8	78.0	234.0	2.99	34	93.1	70.0	172.0	1.83	55	57.2	32.0	74.0	1.03	114	76.4	32.0	234.0	2.58
	Sep-Oct	6	88.5	75.0	93.0	0.69	17	102.8	71.0	238.0	3.8	8	65.9	54.0	75.0	0.69	31	90.5	54.0	238.0	3.20
	Nov-Dec						2	101.5	96.0	107.0	0.85	3	30.0	28.0	31.0	0.12	5	58.6	28.0	107.0	3.84
	Total	42	93.6	75.0	234.0	2.43	65	96.0	70.0	238.0	2.61	104	55.3	23.0	75.0	1.19	211	75.5	23.0	238.0	2.81

In Drepano station, females were the most abundant (N=146; 46.6%), while unsexed individuals the least (N=71; 22.7%) (Table 2.3). The population composed of specimens from 28.0 mm (juvenile) to 253.0 mm (female). At the same time, in Neochori station most individuals were unsexed (N=104; 49.3%) and the least were males (N=42; 30.8%). The shortest total length (23.0 mm) was recorded in a juvenile individual while the longest (238.0 mm) in a male (Table 2.3). The total length of the individuals of the two stations was statistically different (Mann-Whitney test: U=46.712, $p<0.001$) with individuals from Drepano (mean TL= 95.9 mm, Std. error=0.20) being longer than Neochori (mean TL= 75.5 mm, Std. error=0.19).

In the Neochori station, the mean body size of males, females and unsexed individuals was 93.6 mm (St. error=0.38), 96.0 mm (St. error= 0.32) and 55.3 mm (St. error=0.12) respectively. A bimonthly statistical significant difference was recorded in the total length of females as well as in the total length of the unsexed specimens (Figure 2.12). In particular, Bonferoni correction showed that i) female specimens collected in January-February were longer than those collected in March-April period, and ii) the unsexed specimens collected in November-December were the shortest. On the other hand, statistical differences among males were not found.

In Drepano station the mean total length of males, females and unsexed individuals was 109.0 mm (St. error=0.36), 103.9 mm (St. error= 0.26) and 62.0 mm (St. error=0.13) (Table 2.3). There was not found a statistical difference in the total length among male, female or unsexed individuals (Figure 2.12).

Between the individuals in Drepano and Neochori station, the total length differed significantly for females caught in January- February, March- April and July- August period. The specimens caught in January- February were longer in Neochori, whereas, the individuals caught in March- April and July- August period were longer in Drepano (Figure 2.13). Unsexed individuals caught in March-April were statistically longer in Neochori station than Drepano, while were shorter in May-June and November-December periods (Figure 2.13). For the rest of the months the total length was not significantly different between the individuals in the Drepano and Neochori stations (Figure 2.13).

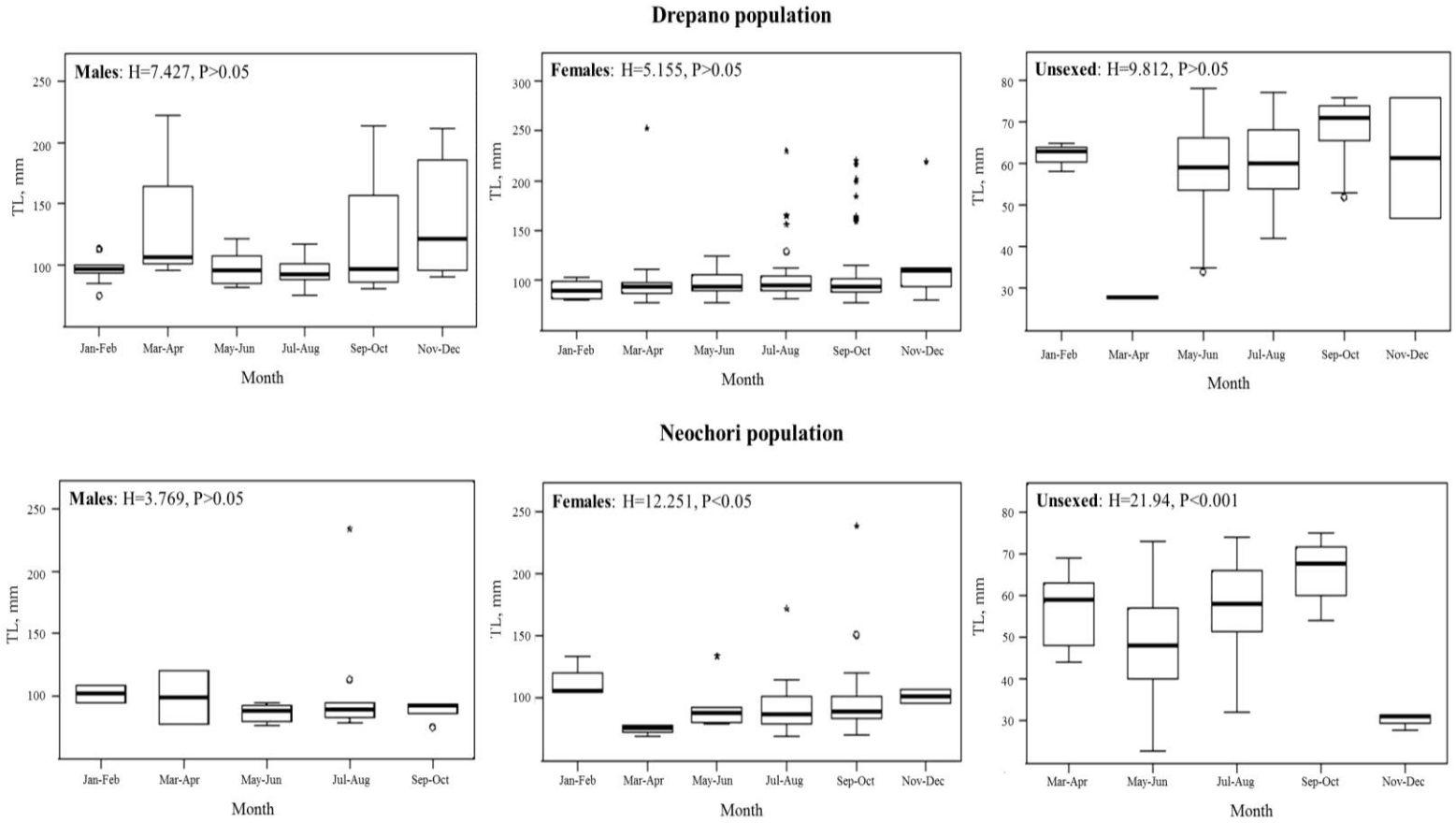

Figure 2.12. Boxplot of the bimonthly variance of total length within the male, female and unsexed specimens of *S. abaster* species caught in Drepano and Neochori stations in the present study (*H*, Value of Kruskal-Wallis non parametric test; *P*, level of significance, the middle bold black line within each box corresponds to the median, the points are outliers, the asterisks are extreme outliers, the T-bars that extend from the boxes correspond to the standard variation).

Εικόνα 2.12. Θηκόγραμμα της διμηνιαίας διακύμανσης του ολικού μήκους των αρσενικών, θηλυκών και αδιευκρίνιστου φύλου ατόμων του είδους *S. abaster* τα οποία συλλέχθηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της παρούσας μελέτης (*H*, η τιμή του μη-παραμετρικού τεστ Kruskal-Wallis; *P*, επίπεδο σημαντικότητας, η μεσαία έντονη γραμμή κάθε θηκογράμματος αντιστοιχεί στο διάμεσο, οι κύκλοι σε ακραίες τιμές, οι αστερίσκοι σε εξαιρετικά ακραίες τιμές και οι γραμμές σχήματος «Τ» στην τυπική απόκλιση του δείγματος).

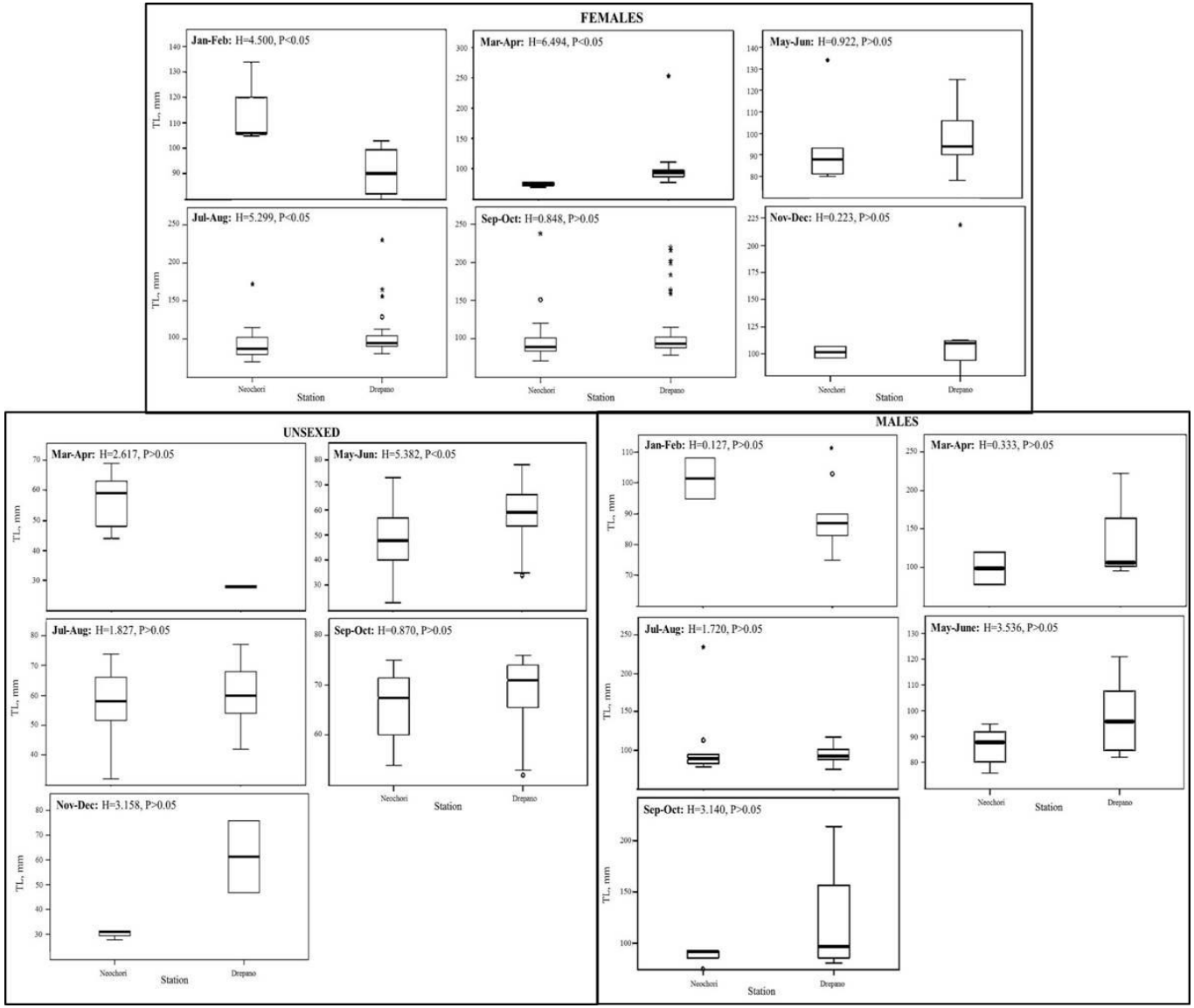

Figure 2.13. Boxplot of the bimonthly variation of total length (TL, mm) among male, female and unsexed specimens of *S. abaster* species caught in Drepano and Neochori stations in the present study (*H*, Value of Kruskal-Wallis non parametric test; *P*, level of significance, the middle bold black line within each box corresponds to the median, the points are outliers, the asterisks are extreme outliers and the T-bars that extend from the boxes correspond to the standard deviation).

Εικόνα 2.13. Θηκόγραμμα της διμηνιαίας διακύμανσης του ολικού μήκους των αρσενικών, θηλυκών και αδιευκρίνιστου φύλου ατόμων του είδους *S. abaster* τα οποία συλλέχθηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της παρούσας μελέτης (*H*, η τιμή του μη- παραμετρικού τεστ Kruskal-Wallis; *P*, επίπεδο σημαντικότητας, η μεσαία έντονη γραμμή κάθε θηκογράμματος αντιστοιχεί στο διάμεσο, οι κύκλοι σε ακραίες τιμές, οι αστερίσκοι σε εξαιρετικά ακραίες τιμές και οι γραμμές σχήματος «Τ» στην τυπική απόκλιση του δείγματος).

2.3.1.2. Sex ratio

The overall sex ratio of *S. abaster* males (N=138) to females (N=211) and adult (N=349) to unsexed (N=175) individuals were statistically different from 1:1 (G- test: $g=15.38$, $p<0.001$ and $g=58.89$, $p<0.001$) (Table 2.4).

In Drepano station, among sexually mature specimens (N=242), females (N=146) outnumbered males (N=96) (G- test: $g=10.41$ $p<0.001$) (Table 2.5). More specifically, sex ratio was statistically significant male biased in January- February and female biased in September-October period (Tables 2.5, 2.3 in Appendix). Adults outnumbered unsexed individuals during the whole study and this difference was statistically significant (G- test: $g=98.73$, $p<0.001$) (Tables 2.5, 2.3 in Appendix).

In Neochori station, among sexually mature specimens (N=107), females (N=65) outnumbered males (N=42), but this difference was statistically important only at the end of the reproductive period (G- test; $g=4.98$, $p<0.05$) (Tables 2.5, 2.3 in Appendix). On the other hand, the sex ratio of adult to unsexed specimens varied during the present study (G-test; $g=0.043$, $p>0.05$). Unsexed individuals were more abundant than adults in May-June period and less abundant in September-October (Tables 2.3, 2.4 in Appendix).

Table 2.4. Number of individuals and overall sex ratio of males to females and adult to unsexed individuals of *S. abaster* species caught in Drepano and Neochori stations in the present study (* level of significance p<0.05).
Πίνακας 2.4. Αριθμός ατόμων και συνολική αναλογία φύλλων αρσενικών προς θηλυκών και ενήλικων προς αδιευκρίνιστου φύλου ατόμων του είδους *S. abaster* τα οποία συλλέχθηκαν από τους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της παρούσας μελέτης (*επίπεδο σημαντικότητας p<0.05).

Males N	Females N	Adult N	Unsexed N	Males:Females	G- test	Adult:Unsexed	G- test
138	211	349	175	1:1.52*	g=15.38, p<0.001	1.99:1*	g=58.89, p<0.001

Table 2.5. Number of individuals and bimonthly sex ratio of male to female and adult to unsexed specimens of *S. abaster* species caught in Drepano and Neochori stations in the present study (* level of significance <0.05).
Πίνακας 2.5. Αριθμός ατόμων και διμηνιαία αναλογία φύλων των αρσενικών προς θηλυκών και ενήλικων προς αδιευκρίνιστου φύλου ατόμων του είδους *S. abaster* τα οποία συλλέχθηκαν από τους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της παρούσας μελέτης (*επίπεδο σημαντικότητας p<0.05).

Station	Month	Males N	Females N	Adult N	Unsexed N	Males: Females	G- test	Adult:Unsexed	G- test
Drepano	Jan-Feb	9	4	13	3	2.25:1*	g=10.41 p<0.001	4.33:1*	g=98.73, p<0.001
	Mar-Apr	3	10	13	1	1:3.33		13:1*	
	May-Jun	19	23	42	20	1:1.21		2.1:1*	
	Jul-Aug	23	25	48	26	1:1.09		1.85:1*	
	Sep-Oct	35	75	110	19	1:2.14*		5.78:1*	
	Nov-Dec	7	9	16	2	1:1.28		8:1*	
Neochori	Jan-Feb	2	3	5	0	1:1.5	g=4.98, p<0.05		g=0.043, p>0.05
	Mar-Apr	2	4	6	13	1:2		1:2.17	
	May-Jun	7	5	12	25	1.4:1		1:2.08*	
	Jul-Aug	25	34	59	55	1:1.36		1.07:1	
	Sep-Oct	6	17	23	8	1:2.83*		2.88:1*	
	Nov-Dec	0	2	2	3				

2.3.1.3. Operational sex ratio

Brooding males of *S. abaster* species were found both in Neochori and Drepano stations from May until the end of October, signaling the duration of the reproductive period. They represented 44.20% (N= 61) of all captured adult males of *S. abaster* species. During the breeding season, their percentage was 50.83%.

In Drepano station, brooding males (N=38) were more abundant than non-brooding (N=39) in the beginning (May-June) and the middle (June-August) of the reproductive season (Table 2.6). In the end of the reproductive season (September-October) non-brooding males prevailed (Table 2.6). The ratio of brooding to non-brooding males ranged from 1:13 to 2.18:1 and was statistically significant during the May-June and September-October periods (Tables 2.6, 2.4 in Appendix). However, the overall ratio did not deviate from the unbiased 1:1 (G- test: $g=0.013$, $p>0.05$). The mean total length of brooding and non-brooding males varied along the reproductive period (Table 2.7). However, a statistically significant difference was not recorded ($N=77$, $p>0.05$) (Table 2.8a).

The operational sex ratio (OSR) was estimated as the ratio of non-brooding to active female individuals across the breeding period. The ratio varied from 1:1.25 to 1:4.6 with non-brooding males being always outnumbered by females (Tables 2.6, 2.4 in Appendix). Therefore, the OSR of individuals in Drepano station was female-biased (G-test: $g=45.75$, $p<0.001$). However, a statistically significant difference was not recorded between the total length of non-brooding males and females (Table 2.8b).

In Neochori station, brooding males (N=23) were more abundant than non-brooding (N=15) along the reproductive period (Table 2.6). The ratio of brooding to non-brooding males ranged from 1:1 to 1:1.6. However, this difference was not statistical significant (Table 2.3 in Appendix) and the overall ratio of brooding to non-brooding males remained unbiased (G- test: $g=1.70$, $p>0.05$). Even though their mean total length varied in a bimonthly basis (Table 2.7), it was not a statistically significant difference (Table 2.8.a).

The operational sex ratio varied from 1:3.4 to 1:5.67 with non-brooding males being always outnumbered by females (Table 2.6). Therefore, the OSR of Neochori was female biased throughout the reproductive season (G- test: $g=25.21$, $p<0.001$). Similarly to the population of Drepano, no statistically significant differences were recorded in the total length of non-brooding males and females (Tables 2.7, 2.8b).

Table 2.6. Number of female, brooding and non-brooding male specimens as well as ratio of brooding to non-brooding males and non-brooding to female individuals of *S. abaster* species caught in Drepano and Neochori stations in the breeding period of the present study (* level of significance <0.05).
Πίνακας 2.6. Αριθμός θηλυκών, κυοφορούντων και μη- κυοφορούντων αρσενικών ατόμων καθώς και η αναλογία κυοφορούντων ως προς μη-κυοφορούντων αρσενικών ατόμων και κυοφορούντων αρσενικών ως προς θηλυκών ατόμων του είδους *S. abaster* τα οποία συλλέχθηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά την αναπαραγωγική περίοδο της παρούσας μελέτης (*επίπεδο σημαντικότητας < 0.05).

Station	Month	Males		Females	Non-brooding: Brooding males	G- test	Non-brooding males:Females	G- test
		Non-brooding	Brooding					
		N	N	N				
Drepano	May-Jun	5	14	23	1:2.8*	g=0.013, p>0.05	1:4.6*	g=45.75, p<0.001
	Jul-Aug	10	13	25	1:1.3		1:2.5*	
	Sep-Oct	24	11	75	2.18:1*		1:3.13*	
Neochori	May-Jun	1	6	5	1:6	g=1.70, p>0.05	1:5*	g=25.21, p<0.001
	Jul-Aug	11	14	34	1:1.27		1:3.4*	
	Sep-Oct	3	3	17	1:1		1:5.67*	

Table 2.7. Mean total length (TL mm) for the bimonthly samples of brooding and non-brooding males and females of *S. abaster* species caught in Drepano and Neochori stations in the breeding period of the present study (*N*, number of individuals; *MTL*, mean total length*; Min*, minimum total length; *Max*, maximum total length*; St.Dev*, standard deviation of total length)

Πίνακας 2.7. Μέσο ολικό μήκος (TL mm) των διμηνιαίων δειγμάτων των κυοφορούντων και μη-κυοφορούντων αρσενικών και θηλυκών ατόμων του είδους *S. abaster* τα οποία συλλέχθηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά την αναπαραγωγική περίοδο της παρούσας μελέτης (*N*, αριθμός ατόμων; *MTL*, μέσο ολικό μήκος*; Min*, ελάχιστο ολικό μήκος; *Max*, μέγιστο ολικό μήκος*; St.Dev*, τυπική απόκλιση ολικού μήκους).

		Non- Brooding Males					**Brooding males**					**Females**				
Station	**Month**	**N**	**MTL**	**Min**	**Max**	**St.Dev**	**N**	**MTL**	**Min**	**Max**	**St.Dev**	**N**	**MTL**	**Min**	**Max**	**St.Dev**
Drepano	Jan-Feb	9	96.8	75.0	113.0	1.21						4	90.8	80.0	103.0	1.06
	Mar-Apr	3	141.3	96.0	222.0	7.00						10	108.4	77.0	253.0	5.16
	May-Jun	5	93.5	82.0	111.0	1.16	14	99.3	84.0	121.0	1.38	23	98.1	78.2	125.1	1.27
	Jul-Aug	10	97.0	85.0	117.0	1.05	13	92.6	75.0	114.0	1.08	25	107.4	81.0	230.0	3.28
	Sep-Oct	24	120.3	81.0	214.0	4.81	11	114.8	85.0	176.0	3.39	75	103.3	78.0	220.0	3.15
	Nov-Dec	7	140.7	90.0	211.0	5.16						9	114.7	80.0	219.0	4.09
	Total	58	114. 9	75.0	222.0	4.13	38	102.2	75.0	176.0	2.23	146	103.9	77.0	253.0	3.14
Neochori	Jan-Feb	2	102.0	95.0	109.0	0.9						3	115.0	105.0	134.0	1.65
	Mar-Apr	2	98.5	77.0	120.0	3.04						4	75.3	70.0	78.0	0.38
	May-Jun	1	76.0	76.0	76.0		6	87.8	76.0	95.0	0.71	5	95.2	80.0	134.0	2.23
	Jul-Aug	11	102.4	79.0	234.0	4.41	14	90.6	78.0	113.0	1.11	34	93.1	70.0	172.0	1.91
	Sep-Oct	3	92.3	91.0	93.0	0.12	3	84.7	75.0	93.0	0.91	17	102.8	71.0	238.0	3.95
	Nov-Dec											2	101.5	96.0	107.0	0.78
	Total	19	90. 5	76.0	234.0	3.44	23	87. 7	75.0	93.0	0.98	65	97.2	70.0	78.0	2.61

Table 2.8. Bimonthly results on the non-parametric Kruskal-Wallis test for the variance of total length between a) brooding and non-brooding and b) non- brooding male and female specimens of *S. abaster* species caught in Drepano and Neochori stations in the breeding period of the present study (N, number of specimens; *H*, Value of Kruskal-Wallis non parametric test; *p*, level of significance).
Εικόνα 2.8. Διμηνιαία αποτελέσματα του μη παραμετρικού ελέγχου Kruskal-Wallis για τη διακύμανση του ολικού μήκους των a) κυοφορούντων και μη αρσενικών και b) μη-κυοφορούντων αρσενικών και θηλυκών ατόμων του είδους *S. abaster* τα οποία συλλέχθηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά την αναπαραγωγική περίοδο της παρούσας μελέτης (*N*, ο αριθμός των ατόμων; *H*, η τιμή του μη- παραμετρικού τεστ Kruskal-Wallis; *P*, επίπεδο σημαντικότητας).

a)

Station	Month	N	H	P-values
Drepano	May-Jun	19	0.549	p>0.05
	Jul-Aug	23	0.652	p>0.05
	Sep-Oct	35	0.248	p>0.05
Neochori	May-Jun	7	1.591	p>0.05
	Jul-Aug	25	0.218	p>0.05
	Sep-Oct	6	1.344	p>0.05

b)

Station	Month	N	H	P-values
Drepano	May-Jun	37	0.706	p>0.05
	Jul-Aug	36	0.262	p>0.05
	Sep-Oct	86	0.341	p>0.05
Neochori	May-Jun	11	2.143	p >0.05
	Jul-Aug	48	0.157	p>0.05
	Sep-Oct	20	0.025	p>0.05

2.3.1.4. Length classes

The bimonthly length frequency distribution of adult and unsexed specimens of Drepano and Neochori stations is shown in Figure 2.14. In both stations, there were revealed two length classes indicating the occurrence of two cohorts per year. The first cohort constitutes mainly of unsexed and adult individuals smaller than 120 mm (0+), while the second cohort consisted only adult individuals larger than 120 mm (1+).

In the station of Drepano, the first cohort was in high abundances every month. Unsexed specimens were present all year long (Figure 2.14). The smallest unsexed individuals (less than 50 mm) appeared in the March-April (March) and November-December (March) periods. High abundances of this group were recorded from May until

August. Adult individuals were also present throughout the year and in higher abundance than recruits (Figure 2.14). The second cohort was observed in low abundances in the March- April period and from July till December (Figure 2.14).

In the Neochori station, the first cohort was in high all year long. Unsexed specimens were present from the March-April period (April month) until (November month) (Figure 2.14). The smallest unsexed individuals (less than 50 mm) were recorded in the November-December period (November month). High abundances of this group were recorded from April until August. Adult individuals were also present throughout the year however their abundance was not always higher than the unsexed (Figure 2.14). The second cohort was less obvious than in the Drepano station. Yet individuals exceeding the 125 mm were recorded in the May-June and September-October periods (Figure 2.14).

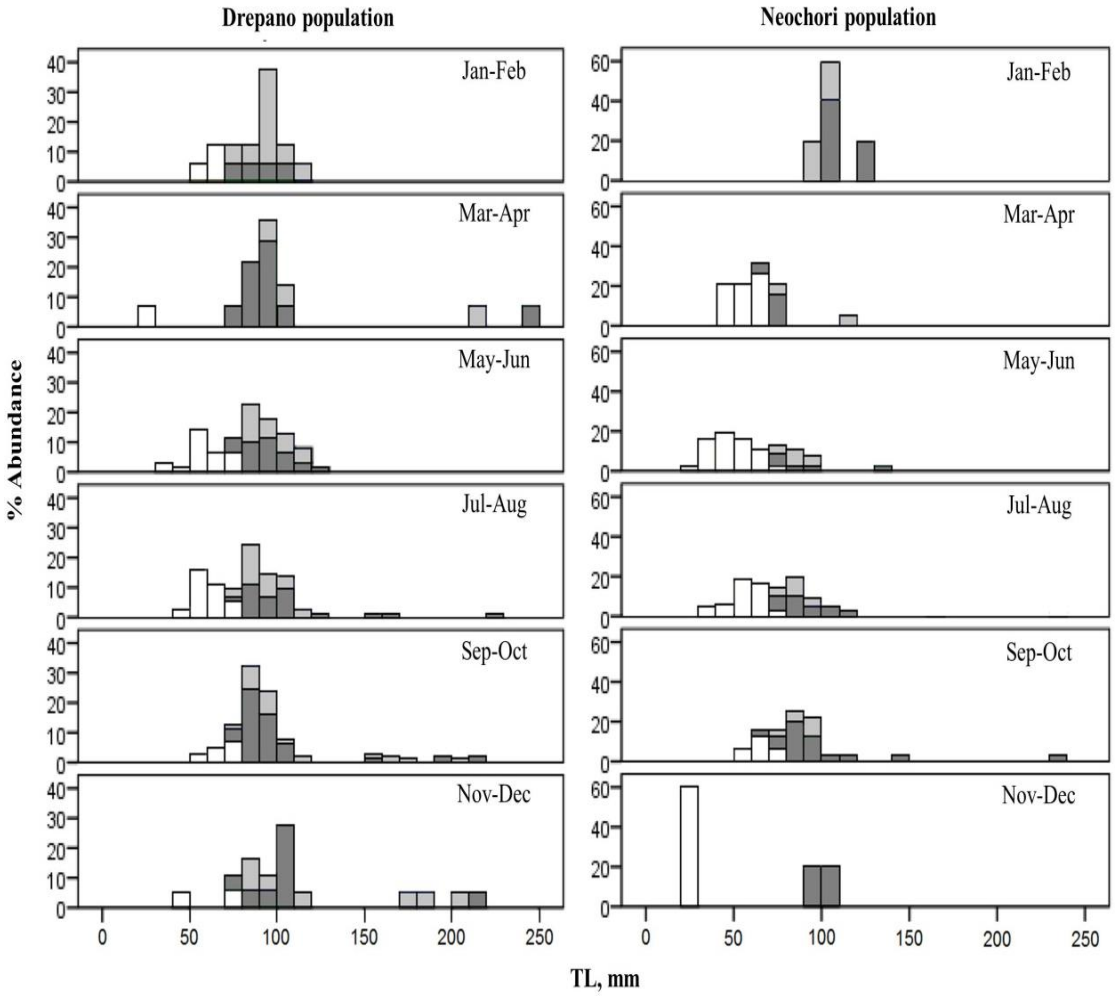

Figure 2.14. Per cent frequency distributions of total length (mm) of *S. abaster* species caught in Drepano and Neochori stations in the present study (unsexed specimens: white colored; males: light gray colored; Females: dark grey- colored).

Εικόνα 2.14. Ποσοστιαία συχνότητα κατανομής του ολικού μήκους (mm) των ατόμων του είδους *S. abaster* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη παρούσα μελέτη (αδιευκρίνιστου φύλου άτομα: λευκό χρώμα; αρσενικά: ανοιχτό γκρι; Θηλυκά: σκούρο γκρι).

2.3.1.5. Size at maturity (L_{50})

Logistic regression model estimating L_{50} correctly classified 97.6% of males (Hosmer and Lemeshow goodness of fit test; d.f. = 8, $p > 0.05$) and 99.1% of females (Hosmer and Lemeshow goodness of fit test; d.f. = 6, $p > 0.05$) from Drepano station (Table 2.9). At the same time, males from Neochori station were 99.3% correctly classified (Hosmer and Lemeshow goodness of fit test; d.f. = 7, $p > 0.05$) and females were 96.4% (Hosmer and Lemeshow goodness of fit test; d.f. = 8, $p > 0.05$) (Table 2.9).

Lengths at 50% maturity (L_{50}) of males and females of *S. abaster* species from Drepano station did not vary (75.5 mm and 75.0 mm respectively) (Figure 2.15). However, the slope of logistic equation was steeper in females than males. Therefore, females in Drepano station reach L_{50} faster than males, yet this difference was not statistically significant (Figure 2.15). In Neochori station, L_{50} for both species did not differ significantly (males: 70.5 mm; females: 72.3 mm) (Figure 2.15). The steeper (almost vertical) slope of the males logistic curve indicate that they reach L_{50} faster than females, however this difference was not statistically important (Figure 2.15).

Table 2.9. Classification results for the cross-validation procedure for the logistic regression model estimating L_{50} of male and female specimens of *S. abaster* species caught in Drepano and Neochori stations in the present study.
Πίνακας 2.9. Επανατοποθέτηση των αποτελεσμάτων του μοντέλου λογιστικής παλινδρόμησης για την εκτίμηση του L_{50} των αρσενικών και θηλυκών ατόμων του είδους *S. abaster* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου στην παρούσα μελέτη.

Station	Sex	Recorded sex status	Predicted sex status			
			Immature	Mature	Percentage	Nagelkerke R^2
Drepano	Males	Immature	70	1	98.6	0.957
		Mature	3	93	96.9	
		Overall			97.6	
	Females	Immature	70	1	98.6	0.985
		Mature	1	145	99.3	
		Overall			99.1	
Neochori	Males	Immature	100	1	99.0	0.992
		Mature	0	42	100.0	
		Overall			99.3	
	Females	Immature	98	3	97.0	0.938
		Mature	3	62	95.4	
		Overall			96.4	

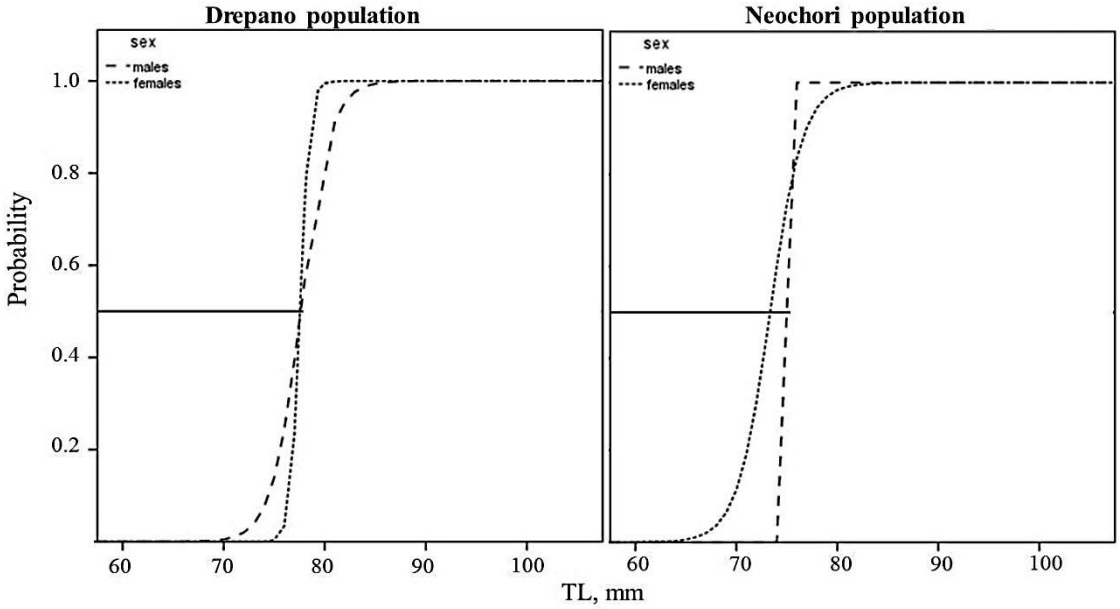

Figure 2.15. Logistic regression curves for the estimation of the L_{50} for male and female individuals, of *S. abaster* species caught in Drepano and Neochori stations during the present study (The interception of the horizontal line and the regression curve correspond to the L_{50}) (Table 2.5 in Appendix).

Εικόνα 2.15. Καμπύλη λογιστικής παλινδρόμησης για την εκτίμηση του L_{50} των αρσενικών και θηλυκών ατόμων του είδους *S. abaster* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου στην παρούσα μελέτη (Το σημείο τομής της οριζόντιας γραμμής και της σιγμοειδής καμπύλης αντιστοιχεί στο L_{50}) (Table 2.5 του Παραρτήματος).

2.3.1.6. Length-weight relationship (LWR)

The length-weight relationship (LWR) was estimated for male, female and unsexed individuals of *S. abaster* species during the months with the highest abundances, i.e. reproductive season (May-October) (Table 2.10, Figure 2.16). ANCOVA analysis indicated that among the examined parameters, sex and periods were the ones affecting the LWR (Table 2.11). In particular, the most important were: i) the interaction of sex and total length (explaining 10.1% of total variance), ii) sex (9.8%), iii) the interaction of station, sex and total length (4.3%), iv) the interaction of sex, month and total length (3.9%), iv) month (2%), and vi) the interaction of month and total length (1.8%) (Table 2.11).

In both stations males and females had higher b-values than unsexed specimens (Table 2.10, Figure 2.16). Based on the estimated b- values, males and females in both stations exhibited positive allometric growth pattern, while unsexed specimens isometric pattern (Figure 2.16, Table 2.10).

Table 2.10. Parameters of the length-weight relationship for male, female and unsexed individuals of *S. abaster* species caught in Drepano and Neochori stations during the reproductive period of the present study (*N*, number of individual; *CI*, confidence interval; + positive allometric growth pattern; =, isometric growth pattern).

Πίνακας 2.10. Συντελεστές της σχέσης Μήκους- Βάρους των αρσενικών, θηλυκών και αδιευκρίνιστου φύλου ατόμων του είδους *S. abaster* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά την αναπαραγωγική περίοδο της παρούσας μελέτη (*N*, αριθμός των ατόμων; *CI*, διάστημα εμπιστοσύνης; + θετικά αλλομετρικη αύξηση; =, ισομετρική αύξηση).

Station	Sex	Month	N	a		b		R^2	Allometry	
				Value	± 95% CI	Value	± 95% CI		Type	P values
Drepano	Males	May-Jun	19	0.98	0.01	3.76	0.48	0.97	+	<. 0.05
		Jul-Aug	23	0.93	0.01	3.11	0.54	0.97	=	<. 0.05
		Sep-Oct	35	0.98	0.04	3.63	0.15	0.98	+	<. 0.001
	Females	May-Jun	23	0.95	0.01	3.25	0.20	0.96	+	<. 0.05
		Jul-Aug	25	0.96	0.02	3.41	0.26	0.94	+	<. 0.05
		Sep-Oct	75	0.97	0.03	3.59	0.20	0.97	+	<. 0.001
	Unsexed	May-Jun	20	0.88	0.03	2.87	0.26	0.95	=	<. 0.001
		Jul-Aug	26	0.87	0.02	2.70	0.29	0.99	=	<. 0.001
		Sep-Oct	19	0.88	0.04	2.63	0.39	0.93	=	<. 0.05
Neochori	Males	May-Jun	7	0.95	0.01	3.33	1.45	0.89	=	> 0.05
		Jul-Aug	25	0.95	0.03	3.34	0.19	0.97	+	<. 0.001
		Sep-Oct	6	0.99	0.01	4.34	1.01	0.86	+	>. 0.05
	Females	May-Jun	5	0.97	0.00	3.50	0.44	0.89	+	> 0.05
		Jul-Aug	34	0.96	0.03	3.37	0.22	0.96	+	<. 0.001
		Sep-Oct	17	0.96	0.03	3.41	0.21	0.96	+	<. 0.05
	Unsexed	May-Jun	25	0.87	0.03	2.69	0.23	0.98	=	<. 0.05
		Jul-Aug	55	0.89	0.05	2.99	0.15	0.99	=	<. 0.001
		Sep-Oct	8	0.88	0.04	2.97	0.19	0.91	=	>. 0.05

Table 2.11. Factors affecting the length- weight relationship of *S. abaster* species caught in Drepano and Neochori stations as shown by the results of the ANCOVA analysis in the present study (*F*, values of significance test; *P-values*, level of significance *, interaction).
Πίνακας 2.11. Παράγοντες που επηρεάζουν τη Σχέση Μήκους-Βάρους του είδους *S. abaster* σύμφωνα με τα αποτελέσματα της Ανάλυσης Διακύμανσης, από άτομα τα οποία συλλέχτηκαν στους σταθμούς το Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της παρούσας μελέτης (*F*, τιμές ελέγχου σημαντικότητας; *P-values*, επίπεδο σημαντικότητας, * αλληλεπίδραση).

Species	**Factor**	**F**	**P- values**	**Partial Eta Squared**	**R^2**
S. abaster	TL	9354.887	0	0.914	0.974
	Station	1.084	0.298	0.001	
	Sex	47.64	0	0.098	
	Month	2.972	0.007	0.02	
	Station * TL	1.125	0.289	0.001	
	Sex *TL	49.127	0	0.101	
	Month *TL	2.74	0.012	0.018	
	Station * Sex * TL	19.672	0	0.043	
	Station * Month * TL	1.209	0.299	0.008	
	Sex * Month * TL	2.969	0	0.039	

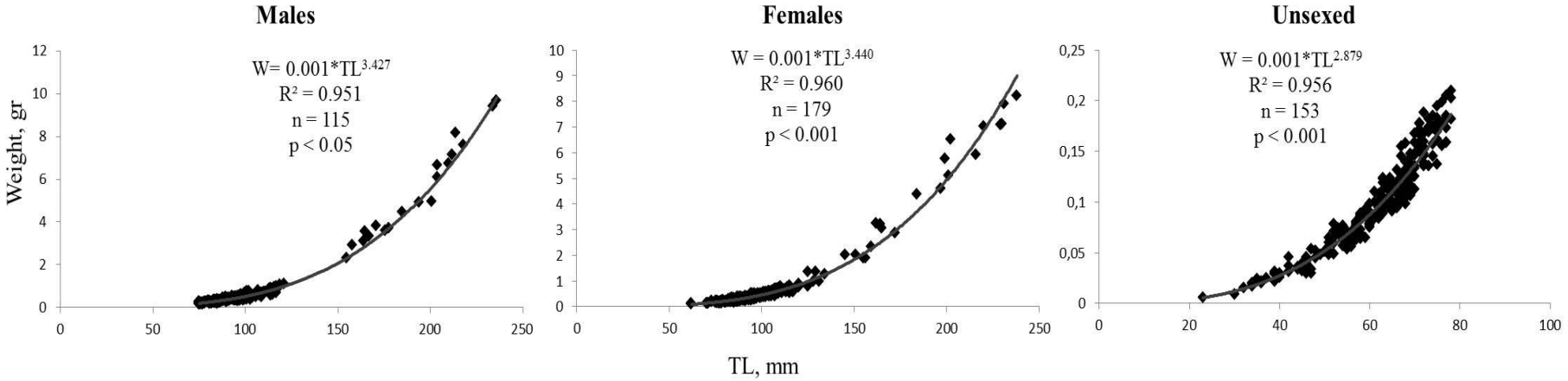

Figure 2.16. Total length-total weight relationship (mm, gr) of male, female and unsexed individuals of *S. abaster* species caught in Drepano and Neochori stations during the reproductive period of the present study.

Εικόνα 2.16. Σχέση ολικού μήκους-ολικού βάρους (mm, gr) αρσενικών, θηλυκών και αδιευκρίνιστου φύλου ατόμων του είδους *S. abaster* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της αναπαραγωγικής περιόδου της παρούσας μελέτης.

2.3.1.7. Gonadosomatic Index (GSI)

Values of gonadosomatic index were higher in females than males in both stations (Figure 2.17). In Drepano station, GSI of both sexes peaked in March-April period. The lowest values for both sexes were recorded in November-December. The GSI of males and females followed the same fluctuation pattern (Figure 2.17).

In Neochori station, the period with the highest values of GSI for females and males did not coincide. Males' index reached peak in May-June period while females' in March- April. The GSI pattern of the two sexes was antisymetrical i.e. when it rose for females it fell for males and vice versa (Figure 2.17).

ANCOVA analysis showed that sex (15.3%), month-period (7.7 %), station (1.5%) and the interaction of sex- moth and station-month-sex were the statistically significant factors that influenced the GSI. On the other hand, the interactions of station-sex and station-month were not significant (Table 2.12).

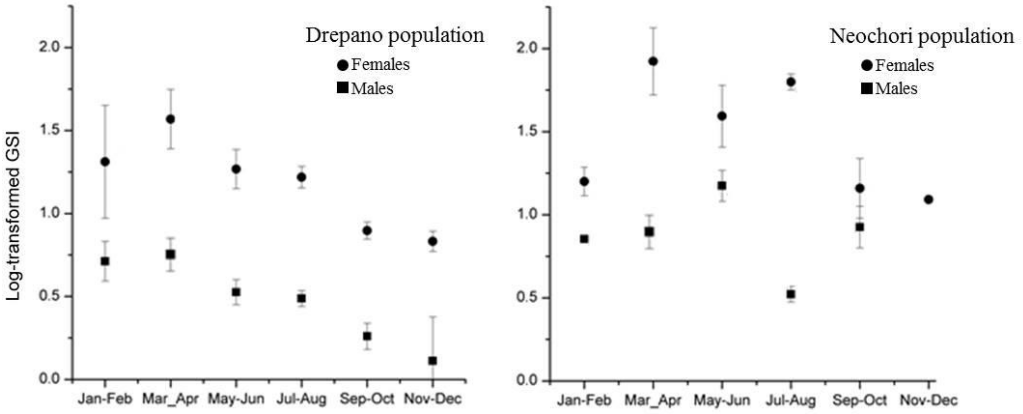

Figure 2.17. Error bars of the variation of gonadosomatic index (GSI) for male and female individuals of *S. abaster* species caught in Drepano and Neochori stations during the present study (T-bars that extend from the points correspond to the standard deviation).
Εικόνα 2.17. Διαγράμματα σφαλμάτων της διακύμανσης του γοναδοσωματικού δείκτη (ΓΣΔ) των αρσενικών και θηλυκών ατόμων του είδους *S. abaster*, τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη παρούσα μελέτη (οι γραμμές σχήματος «Τ» αντιστοιχούν στην τυπική απόκλιση του δείγματος).

Table 2.12. Factors affecting the values of gonadosomatic index (GSI) of *S. abaster* species as shown by the results of the ANCOVA analysis. The specimens used in the present study were caught in Drepano and Neochori stations (*F*, values of significance test; *P-values*, level of significance *, interaction).
Πίνακας 2.12. Παράγοντες που επηρεάζουν τον γοναδοσωματικού δείκτη (ΓΣΔ) του είδους *S. abaster* σύμφωνα με τα αποτελέσματα της Ανάλυσης Συνδιακύμανσης. Τα άτομα που χρησιμοποιήθηκαν στη παρούσα μελέτης συλλέχθηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου (*F*, τιμές ελέγχου σημαντικότητας; *P-values*, επίπεδο σημαντικότητας, * αλληλεπίδραση).

Factor	F	P- values	Partial Eta Squared
Sex	67.323	0	0.153
Month	2.823	0.001	0.077
Month * Sex	2.219	0.013	0.061
Station * Month * Sex	4.056	0.001	0.052
Station	5.569	0.019	0.015
Station * Month	0.975	0.449	0.018
Station * Sex	0.019	0.889	0

2.3.1.8. Hepatosomatic Index (HSI)

Values of the hepatosomatic index, generally, were higher in females than males in both stations (Figure 2.18). In Drepano station, the values of HSI of both sexes peaked in the March-April period while the lowest values were recorded in the July- August. The values of HSI displayed limited variance in both sexes (Figure 2.18).

In Neochori station, the highest values of HSI for females were recorded in May-June period, while the lowest in January-February. Males' index reached peak in September-October period and low in May-June. Contrary to Drepano station, the values of HSI displayed high variance in both sexes (Figure 2.18).

ANCOVA analysis showed that sex (6.7%), station (2.5%) and the interaction of sex- moth, station- sex and station-month-sex were the statistically significant factors that influenced the values of the HSI. On the other hand, the month and the interactions of station-month were not statistically significant (Table 2.13).

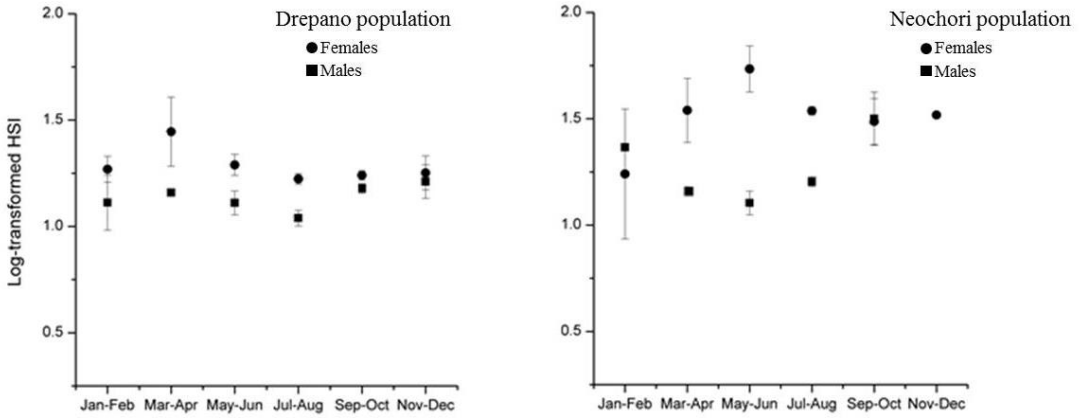

Figure 2.18. Error bars of the variation of the hepatosomatic index (HSI) for male and female individuals of *S. abaster* species collected in Drepano and Neochori stations during the present study (T-bars that extend from the points correspond to the standard deviation).
Εικόνα 2.18. Διαγράμματα σφαλμάτων της διακύμανσης του ηπατοσωματικού δείκτη (ΗΣΔ) των αρσενικών και θηλυκών ατόμων του είδους *S. abaster*, τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη παρούσα μελέτη (οι γραμμές σχήματος «Τ» αντιστοιχούν στην τυπική απόκλιση του δείγματος).

Table 2.13. Factors affecting the values of the hepatosomatic index (HSI) of *S. abaster* species from specimens collected in Drepano and Neochori stations as showed by the results of the ANCOVA analysis in the present study (*F*, values of significance test; *P-values*, level of significance *, interaction).
Πίνακας 2.12. Παράγοντες που επηρεάζουν τον ηπατοσωματικό δείκτη (ΗΣΔ) του είδους *S. abaster* στα άτομα που συλλέχθηκα στους σταθμούς του Δρεπάνου και του Νεοχωρίου σύμφωνα με τα αποτελέσματα της Ανάλυσης Συνδιακύμανσης της παρούσας μελέτης (*F*, τιμές ελέγχου σημαντικότητας; *P-values*, επίπεδο σημαντικότητας, * αλληλεπίδραση).

Factor	F	P- values	Partial Eta Squared
Month * Sex	2.775	0.002	0.076
Sex	26.799	0	0.067
Station * Month * Sex	3.441	0.005	0.044
Station	9.705	0.002	0.025
Station * Sex	6.96	0.009	0.018
Month	1.47	0.141	0.042
Station* Month	0.987	0.44	0.018

2.3.1. *Syngnathus typhle* species

2.3.2.1. Population structure

A total of 724 specimens of *S. typhle* species were caught in Drepano (N=287) and Neochori (N=437) stations during the present study. The relative abundance of sampled specimens was not statistically different between the two stations (Mann- Whitney test: U= 92.500, p> 0.05). The number of sampled individuals between the examined periods was statistically different in Neochori station (Kruskal- Wallis test population: H=12.249, p<0.05), but did not differ in Drepano station (Kruskal- Wallis test: H=10.534, p>0.05) (Figure 2.19). In particular, high abundances in Neochori station were recorded from May until October. (Figure 2.19). The rest of the months were characterized by low numbers of caught individuals (Figure 2.19). Between the two stations the only statistically significant difference in the relative abundance was found in July-August period when the number of Neochori specimens was marginally higher than Drepano's (Kruskal- Wallis test: H= 3.857, p=0.05).

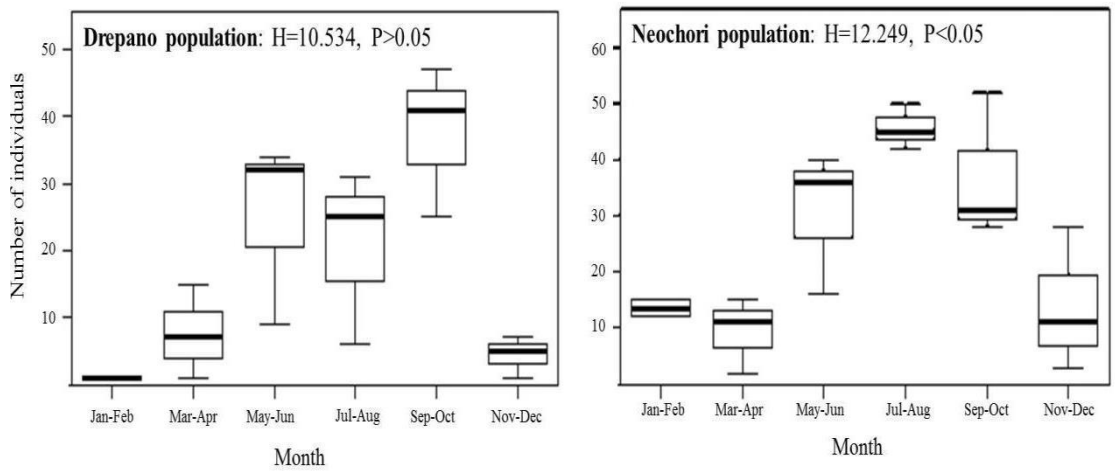

Figure 2.19 Box plot of the average number of individuals of *S. typhle* species caught bimonthly in Drepano and Neochori stations in the present study (*H,* Value of Kruskal-Wallis non parametric test; *P,* level of significance, the middle bold black line within each box corresponds to the median and the T-bars that extend from the boxes correspond to the standard deviation).

Εικόνα 2.19. Θηκόγραμμα του μ.ο. του αριθμού των ατόμων του είδους *S. typhle* τα οποία συλλέχτηκαν ανά δίμηνο στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της παρούσας μελέτης (*H,* η τιμή του μη- παραμετρικού τεστ Kruskal-Wallis; *P,* επίπεδο σημαντικότητας, η μεσαία έντονη γραμμή κάθε θηκογράμματος αντιστοιχεί στο διάμεσο, και οι γραμμές σχήματος «Τ» στην τυπική απόκλιση του δείγματος).

Table 2.14. Mean total length (TL mm) of male, female, unsexed and sexes combined individuals of *S. typhle* species caught in Drepano and Neochori station in the present study (*N*, number of individuals; *MTL*, mean total length*; Min*, minimum total length; *Max*, maximum total length*; St.Dev*, standard deviation of total length)

Πίνακας 2.3. Μέσο ολικό μήκος (TL mm) των αρσενικών, θηλυκών, αδιευκρίνιστου φύλου και για το σύνολο των ατόμων του είδους *S. typhle* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά η διάρκεια της παρούσας μελέτης (*N*, αριθμός ατόμων; *MTL*, μέσο ολικό μήκος*; Min*, ελάχιστο ολικό μήκος; *Max*, μέγιστο ολικό μήκος*; St.Dev*, τυπική απόκλιση ολικού μήκους).

		Males					**Females**					**Unsexed**					**Total**				
Station	**Month**	**N**	**MTL**	**Min**	**Max**	**St. Dev.**	**N**	**MTL**	**Min**	**Max**	**St. Dev.**	**N**	**MTL**	**Min**	**Max**	**St. Dev.**	**N**	**MTL**	**Min**	**Max**	**St. Dev.**
Drepano	Jan-Feb	1	95.0	95.0	95.0		0					0					1	95.0	95.0	95.0	
	Mar-Apr	7	131.1	88.0	172.0	2.53	15	170.1	121.0	243.0	4.12	1	40.0	40.0	40.0		23	152.6	40.0	243.0	4.61
	May-Jun	32	134.6	85.0	237.0	3.44	34	116.1	84.0	193.0	2.70	9	68.0	53.0	78.0	0.95	75	118.2	53.0	237.0	3.51
	Jul-Aug	25	143.0	104.0	214.0	2.54	31	129.7	80.4	194.0	3.01	6	60.5	46.0	70.0	0.92	62	128.4	46.0	214.0	3.48
	Sep-Oct	41	131.5	73.0	214.0	3.41	47	139.2	80.0	220.0	3.72	25	57.1	33.0	78.0	1.07	113	118.2	33.0	220.0	4.48
	Nov-Dec	7	130.6	105.0	155.0	1.49	5	152.6	90.0	207.0	5.34	1	72.0	72.0	72.0		13	134.5	72.0	207.0	3.89
	Total	113	127.6	73.0	237.0	2.68	132	141.54	80.0	243.0	3.78	42	59.52	33.0	78.0	0.98	287	124.48	33.0	243.0	3.99
Neochori	Jan-Feb	12	132.5	101.0	157.0	1.77	15	135.6	102.0	181.0	2.22	0					27	134.2	101.0	181.0	2.00
	Mar-Apr	15	143.2	77.0	185.8	2.88	11	125.8	98.7	151.1	1.18	2	74.5	73.0	76.0	0.21	28	131.4	73.0	185.8	2.80
	May-Jun	40	107.2	67.0	163.0	2.72	16	111.3	78.0	166.0	3.28	36	58.4	42.0	73.0	0.81	92	88.8	42.0	166.0	3.28
	Jul-Aug	45	120.7	88.0	187.0	2.60	50	111.4	78.0	171.0	2.48	42	61.3	35.0	77.0	1.10	137	99.1	35.0	187.0	3.28
	Sep-Oct	28	117.1	80.0	172.0	2.57	52	113.0	74.0	182.0	2.92	31	67.8	41.0	79.0	0.97	111	101.4	41.0	182.0	3.15
	Nov-Dec	11	132.5	86.0	188.0	3.62	28	118.0	77.0	183.0	2.47	3	54.7	25.0	75.0	2.42	42	117.2	25.0	188.0	3.28
	Total	151	125.53	67.0	188.0	2.69	172	119.18	74.0	183.0	2.43	114	63.34	25.0	79.0	1.10	437	112.02	25.0	188.0	2.97

In both stations females were the most abundant (Drepano: N=132; Neochori: N=172) while unsexed the least (Drepano: N=42; Neochori: N=114) (Table 2.14). The population of Drepano composed of specimens from 33.0 mm (unsexed) to 243.0 mm (female). At the same time, in the Neochori station the shortest individual was unsexed (25.0 mm), while the longest male (188.0 mm) (Table 2.14). The total length of the individuals of the two stations was statistically different (Mann-Whitney test: U=80.203, $p<0.001$) with individuals from Drepano (mean TL= 123.8 mm, Std. error=0.25) being longer than Neochori (mean TL= 103.5 mm, Std. error=0.17).

In Drepano station the mean body size of males, females and unsexed individuals was 134.5 mm (St. error= 0.30), 135.5 (St. error= 0.33) and 60.0 mm (St. error=0.18) respectively (Table 2.14). A bimonthly statistical significant difference was found in the total length among females as well as in the total length among unsexed individuals (Figure 2.20). In particular, Bonferoni correction showed that the largest female specimens occurred in March-April period, while the smallest in May-June (Kruskal-Wallis test: H=19.417, $p<0.001$). For unsexed individuals the opposite pattern was observed i.e. larger individuals in May-June and smaller in January-February (Kruskal-Wallis test: H=9.808, $p<0.05$).

In Neochori station the mean body size of males, females, and unsexed individuals was 120.5 mm (St. error=0.24), 116.0 mm (St. error= 0.22) and 62.2 mm (St. error=0.11), respectively (Table 2.14). Total length varied statistically among male, female and unsexed specimens on a bimonthly bases (Figure 2.20). Bonferoni correction indicated that male and unsexed specimens collected in March- April period and females collected in January-February were longer than those collected in the rest of the months (Figure 2.20).

Between the individuals in Drepano and Neochori stations, the total length differed significantly for males sampled in January- February, May-June and July- August periods. Males from Neochori station were longer than Drepano in January-February samples, while in the other two periods individuals in Drepano were longer (Figure 2.21). Female individuals in Drepano station were longer than Neochori in March-April, July-August and September-October periods (Figure 2.21). Unsexed individuals total length varied significantly in May-June and September-October. In the first period, samples from Drepano were longer than Neochori while in September-October the opposite pattern was recorded (Figure 2.21). Within each sex, for the rest of the months, total length was not significantly different between the individuals in Drepano and Neochori stations (Figures 2.21).

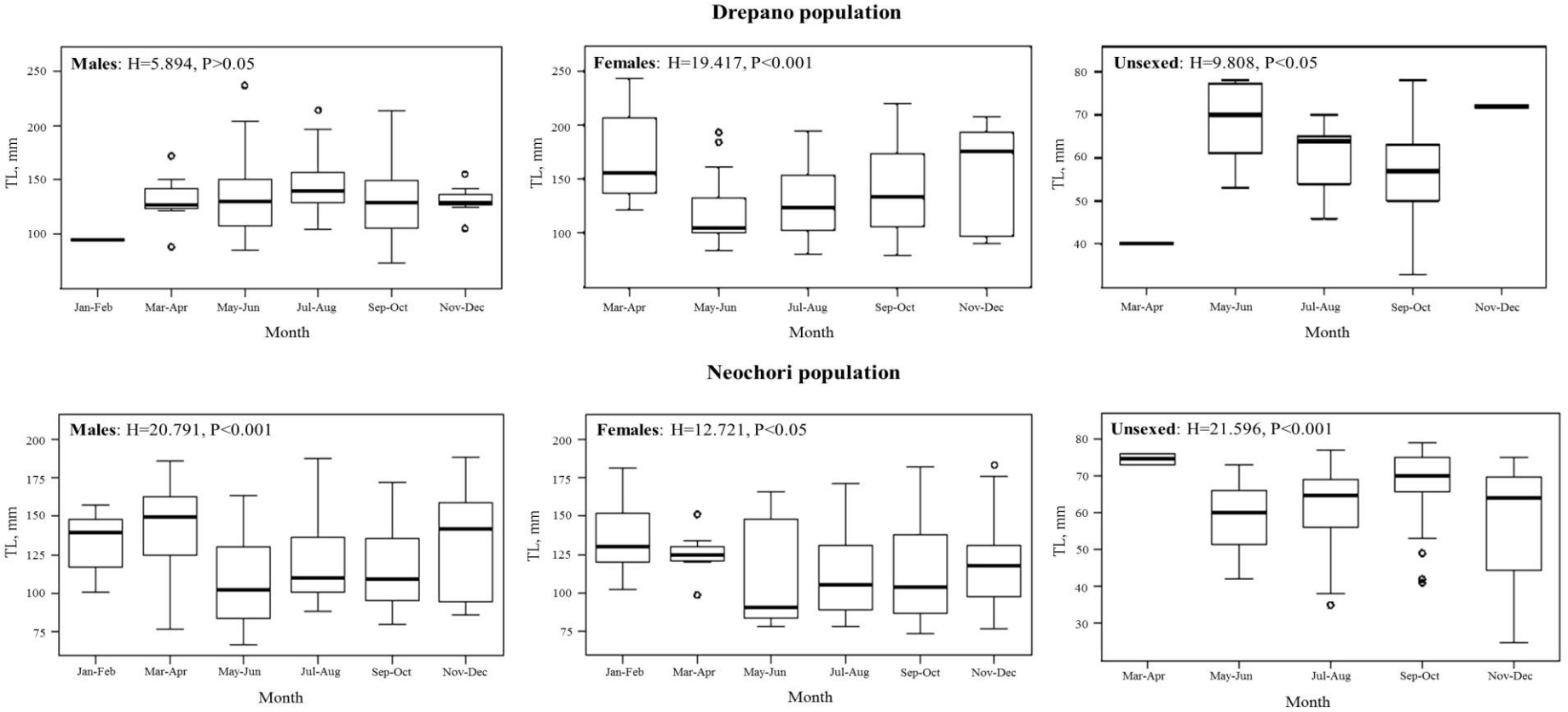

Figure 2.20. Boxplot of the bimonthly variance of total length within the males, females and unsexed specimens of *S. typhle* species caught in Drepano and Neochori stations in the present study (*H,* Value of Kruskal-Wallis non parametric test; *P,* level of significance, the middle bold black line within each box corresponds to the median, the points are outliers, the asterisks are extreme outliers, the T-bars that extend from the boxes correspond to the standard variation).

Εικόνα 2.20. Θηκόγραμμα της διμηνιαίας διακύμανσης του ολικού μήκους των αρσενικών, θηλυκών και αδιευκρίνιστου φύλου ατόμων του είδους *S. typhle* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου, κατά τη διάρκεια της παρούσας μελέτης (*H,* η τιμή του μη-παραμετρικού τεστ Kruskal-Wallis; *P,* επίπεδο σημαντικότητας, η μεσαία έντονη γραμμή κάθε θηκογράμματος αντιστοιχεί στο διάμεσο, οι κύκλοι σε ακραίες τιμές, οι αστερίσκοι σε εξαιρετικά ακραίες τιμές και οι γραμμές σχήματος «Τ» στην τυπική απόκλιση του δείγματος).

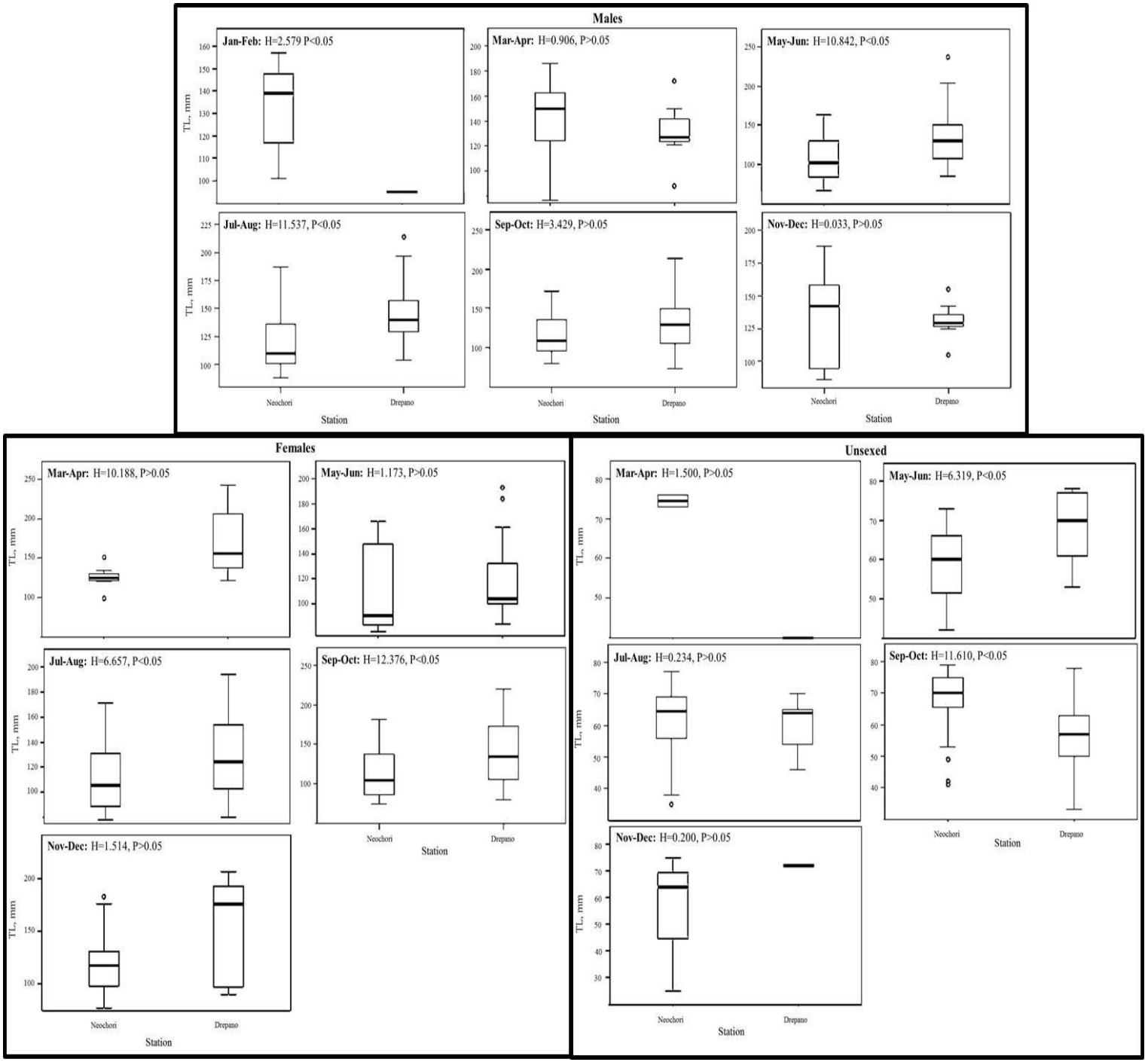

Figure 2.21. Boxplot of the bimonthly variation of total length (TL, mm) among males, females and unsexed specimens of *S. typhle* species caught in Drepano and Neochori stations in the present study (*H*, Value of Kruskal-Wallis non parametric test; *P*, level of significance, the middle bold black line within each box corresponds to the median, the points are outliers, the asterisks are extreme outliers and the T-bars that extend from the boxes correspond to the standard deviation).

Εικόνα 2.21. Θηκόγραμμα της διμηνιαίας διακύμανσης του ολικού μήκους των αρσενικών, θηλυκών και αδιευκρίνιστου φύλου ατόμων του είδους *S. typhle* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της παρούσας μελέτης (*H*, η τιμή του μη- παραμετρικού τεστ Kruskal-Wallis; *P*, επίπεδο σημαντικότητας, η μεσαία έντονη γραμμή κάθε θηκογράμματος αντιστοιχεί στο διάμεσο, οι κύκλοι σε ακραίες τιμές, οι αστερίσκοι σε εξαιρετικά ακραίες τιμές και οι γραμμές σχήματος «Τ» στην τυπική απόκλιση του δείγματος).

2.3.2.2. Sex ratio

The overall the sex ratio of *S. typhle* males (N=264) to females (N=304) remained unbiased (G- test: g=2.82, p>0.05). However, the sex ratio of adult (N=568) to unsexed (N=156) individuals was statistically different from 1:1 with adult specimens being the most abundant (G- test: g=249.11, p<0.001) (Table 2.15).

In Drepano station, among sexually mature specimens (N=245), females (N=132) outnumbered males (N=113) (Table 4.16). However, this difference was not statistically significant in the examined period (G- test; g=1.48, p>0.05). (Tables 2.16, 2.3 in Appendix). The overall ratio of adults to unsexed individuals was adult biased during the present study (G- test: g=158.90 p<0.001) (Tables 2.16, 2.3 in Appendix).

In Neochori station, among sexually mature specimens (N=325), females (N=172) were more abundant than males (N=151) (Table 2.16). This difference was significant in the May- June, September-October and November-December periods while it remained unbiased in the rest of the study (G- test: g=1.37, p>0.05) (Tables 2.16, 2.3 in Appendix). The ratio of adult to unsexed specimens significantly deviated from the unbiased 1:1 ratio (G- test: x^2=104.17, p<0.001) (Tables 2.16, 2.3 in Appendix).

Table 2.15. Number of individuals and overall sex ratio of males to females and adult to unsexed individuals of *S. typhle* species caught in Drepano and Neochori stations in the present study (* level of significance p<0.05).
Πίνακας 2.15. Αριθμός ατόμων και συνολική αναλογία φύλλων αρσενικών προς θηλυκών και ενήλικων προς αδιευκρίνιστου φύλου ατόμων του είδους *S. typhle* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της παρούσας μελέτης (*επίπεδο σημαντικότητας p<0.05).

Males N	Females N	Adults N	Unsexed N	Males:Females	G- test	Adult:Unsexed	G- test
264	304	568	156	1:1.16	g=2.82, p>0.05	3.64:1*	g=249.11, p<0.001

Table 2.16 . Number of individuals and bimonthly sex ratio of male to female and adult to unsexed specimens of *S. typhle* species caught in Drepano and Neochori stations in the present study (* level of significance <0.05).
Πίνακας 2.16. Αριθμός ατόμων και διμηνιαία αναλογία φύλων των αρσενικών προς θηλυκών και ενήλικων προς αδιευκρίνιστου φύλου ατόμων του είδους *S. typhle* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της παρούσας μελέτης (*επίπεδο σημαντικότητας p<0.05).

Station	Month	Males N	Females N	Adults N	Unsexed N	Males:Females	G- test	Adult:Unsexed	G- test
Drepano	Jan-Feb	1	0	1	0		g=1.48, p>0.05		g=158.90 p<0.001
	Mar-Apr	7	15	22	1	1:2.14		22:1*	
	May-Jun	32	34	66	9	1:1.06		7.33:1*	
	Jul-Aug	25	31	56	6	1:1.24		9.33:1*	
	Sep-Oct	41	47	88	25	1:1.15		3.52:1*	
	Nov-Dec	7	5	12	1	1.4:1			
Neochori	Jan-Feb	12	15	27	0	1:1.25	g=1.37 p>0.05		g=104.17, p<0.001
	Mar-Apr	15	11	26	2	1.36:1		13:1*	
	May-Jun	40	16	56	36	2.5:1*		1.56:1*	
	Jul-Aug	45	50	95	42	1:1.11		2.26:1*	
	Sep-Oct	28	52	80	31	1:1.85*		2.58:1*	
	Nov-Dec	11	28	39	3	1:2.54*		13:1*	

2.3.2.3. Operational sex ratio

Brooding males of *S. typhle* species were found both in Neochori and Drepano stations from March-April period until the end of the October, signaling the duration of the species reproductive period. They represented (N= 122) 46.21% of all captured adult males (N=264) of *S. typhle* species. During the breeding season, this percentage rose up to 52.79%.

In Drepano station during the reproductive period, brooding males (N=63) were always more abundant than non-brooding (N=42). The ratio of non-brooding to brooding males ranged from 1:1.13 to 1:1.92 (Table 2.17). However, this difference was statistically significant only in the September- October period (G- test: $g=4.23$, $p<0.05$) (Tables 2.17, 2.4 in Appendix). The mean total length of brooding and non-brooding males varied along the reproductive period (Table 2.18). However, this difference was statistically significant only in the September- October period, with brooding males being longer than non-brooding (Kruskal- Wallis test: $H=5.146$, $p<0.05$ (Table 2.19a).

The operational sex ratio (OSR) was estimated as the ratio of non-brooding to active female individuals across the breeding period. The ratio varied from 1:27 to 1:5 and was significantly female- biased throughout the reproductive period (G- test: $g=44.77$, $p<0.001$) (Table 2.17, 2.4 in Appendix). The total length of non-brooding and female individuals was statistically different in September- October period (Kruskal- Wallis test: H: 4.063, $p<0.05$), with females being longer than non-brooding males (Table 2.18, 2.19b).

In Neochori population, non-brooding males (N=69) outnumbered broodings males (N=59) in all months of the reproductive season except from the July-August period (Table 2.17). The ratio of non-brooding to brooding males ranged from 1:1.65 to 3:1 and it was statistically significant in the July- August and September- October periods (Table 2.17). However, the overall ratio of brooding to non-brooding males did not deviate from the unbiased 1:1 (G- test: $g=0.78$, $p>0.05$) (Tables 2.17, 2.4. in Appendix). The mean total length of brooding and non-brooding males in both stations varied along the reproductive period (Table 2.18). However, this difference was statistically significant only in the begging (Mach-April) and the end (September- October) of the reproductive season, with brooding males being longer than non-brooding (Table 2.19a).

The operational sex ratio of non-brooding to active females individuals across the breeding period varied from 1.38:1 to 1:2.94 and overall was significantly female-biased (G- test: $g=18.47$, $p<0.001$) (Table 2.17). More specifically, it was female biased in July-August period, but it was male biased in the May-June (Tables 2.17, 2.10 in Appendix). Even though the total length of female and non-brooding individuals varied in the reproductive season, this variance was not statistically significant (Table 2.19b).

Table 2.17. Number of female, brooding and non-brooding male specimens as well as ratio of brooding to non-brooding males and non-brooding to female individuals of *S. typhle* species caught in the station of Drepano and Neochori during the breeding period of the present study (* level of significance <0.05).

Πίνακας 2.17. Αριθμός θηλυκών, κυοφορούντων και μη- κυοφορούντων αρσενικών ατόμων καθώς και η αναλογία κυοφορούντων ως προς μη-κυοφορούντων αρσενικών ατόμων και κυοφορούντων αρσενικών ως προς θηλυκών ατόμων του είδους *S. typhle* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά την αναπαραγωγική περίοδο της παρούσας μελέτης (*επίπεδο σημαντικότητας < 0.05).

Station	Month	Males		Females	Non-brooding: Brooding males	G- test	Non-brooding males: Females	G- test
		Non-brooding	Brooding					
		N	**N**	N				
Drepano	Mar-Apr	3	4	15	1:1.33	g=4.23, p<0.05	1:5*	g=44.77, p<0.001
	May-Jun	15	17	34	1:1.13		1:2.27*	
	Jul-Aug	10	15	31	1:1.5		1:3.1*	
	Sep-Oct	14	27	47	1:1.92*		1:3.36*	
Neochori	Mar-Apr	9	6	11	1.5:1	g=0.78, p>0.05	1:1.22	g=18.47, p<0.001
	May-Jun	22	18	16	1.22:1		1.38:1	
	Jul-Aug	17	28	50	1:1.65*		1:2.94*	
	Sep-Oct	21	7	52	3:1*		1:2.48*	

Table 2.18. Mean total length (TL mm) for the bimonthly samples of brooding and non-brooding males and females of *S. typhle* species caught in Drepano and Neochori stations in the breeding period of the present study (*N*, number of individuals; *MTL*, mean total length*; Min*, minimum total length; *Max*, maximum total length*; St.Dev*, standard deviation of total length)

Πίνακας 2.18. Μέσο ολικό μήκος (TL mm) των διμηνιαίων δειγμάτων των κυοφορούντων και μη-κυοφορούντων αρσενικών και θηλυκών ατόμων του είδους *S. typhle* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά την αναπαραγωγική περίοδο της παρούσας μελέτης (*N*, αριθμός ατόμων; *MTL*, μέσο ολικό μήκος*; Min*, ελάχιστο ολικό μήκος; *Max*, μέγιστο ολικό μήκος*; St.Dev*, τυπική απόκλιση ολικού μήκους).

		Non-Brooding Males					**Brooding males**					**Females**				
Station	**Month**	**N**	**MTL**	**Min**	**Max**	**St.Dev**	**N**	**MTL**	**Min**	**Max**	**St.Dev**	**N**	**MTL**	**Min**	**Max**	**St.Dev**
Drepano	Jan-Feb	1	95.0	95.0	95.0											
	Mar-Apr	3	129.0	88.0	172.0	4.20	4	132.8	121.0	150.0	1.27	15	170.1	121.0	243.0	4.21
	May-Jun	15	124.8	85.0	204.0	3.20	17	143.2	105.0	237.0	3.74	34	116.1	84.0	193.0	2.80
	Jul-Aug	10	141.4	104.0	214.0	2.88	15	144.0	105.0	197.0	2.53	31	129.7	80.4	194.0	3.14
	Sep-Oct	14	114.9	73.0	198.0	3.31	27	140.1	97.0	214.0	3.33	47	139.2	80.0	220.0	3.80
	Nov-Dec	7	130.6	105.0	155.0	1.54						5	152.6	90.0	207.0	5.51
	Total	50	122.6	73.0	214.0	3.03	63	140.0	97.0	237.0	2.72	132	170.1	80.0	243.0	3.89
Neochori	Jan-Feb	12	132.5	101.0	157.0	1.85						15	135.6	102.0	181.0	2.31
	Mar-Apr	9	127.4	77.0	169.9	2.80	6	167.0	155.6	185.8	1.13	11	125.8	98.7	151.1	1.25
	May-Jun	22	101.2	67.0	163.0	2.89	18	114.5	81.0	156.0	2.68	16	111.3	78.0	166.0	3.43
	Jul-Aug	17	111.9	88.0	166.0	2.28	28	126.1	94.0	187.0	2.82	50	111.4	78.0	171.0	2.57
	Sep-Oct	21	109.5	80.0	172.0	2.14	7	139.7	93.0	167.0	3.02	52	113.0	74.0	182.0	3.04
	Nov-Dec	11	132.5	86.0	188.0	3.79						28	118.0	77.0	183.0	2.58
	Total	92	119.2	67.0	188.0	2.83	59	136.8	81.0	187.0	3.03	172	119.2	74.0	183.0	2.79

Table 2.19. Bimonthly results on the non-parametric Kruskal-Wallis test for the variance of total length between a) brooding and non-brooding and b) non- brooding male and female specimens of *S. typhle* species caught in Drepano and Neochori stations in the breeding period of the present study (N, number of specimens; *H*, Value of Kruskal-Wallis non parametric test; *p*, level of significance).

Πίνακας 2.19. Διμηνιαία αποτελέσματα του μη παραμετρικού ελέγχου Kruskal-Wallis για τη διακύμανση του ολικού μήκους των a) κυοφορούντων και μη αρσενικών και b) μη-κυοφορούντων αρσενικών και θηλυκών ατόμων του είδους *S. typhle* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά την αναπαραγωγική περίοδο της παρούσας μελέτης (*N*, ο αριθμός των ατόμων; *H*, η τιμή του μη- παραμετρικού τεστ Kruskal-Wallis; *p*, επίπεδο σημαντικότητας).

a)

Station	month	N	H	P-values
Drepano	Mar-Apr	7	0.001	p>0.05
	May-Jun	32	1.798	p>0.05
	Jul-Aug	25	0.443	p>0.05
	Sep-Oct	**41**	**5.146**	p**<0.05**
Neochori	**Mar-Apr**	**17**	**7.374**	p**<0.05**
	May-Jun	40	2.937	p>0.05
	Jul-Aug	45	2.963	p>0.05
	Sep-Oct	**28**	**4.399**	p**<0.05**

b)

Station	Month	N	H	P-values
Drepano	Mar-Apr	18	1.856	p>0.05
	May-Jun	51	1.018	p>0.05
	Jul-Aug	46	1.228	p>0.05
	Sep-Oct	**74**	**4.063**	p**<0.05**
Neochori	Mar-Apr	18	0.036	p>0.05
	May-Jun	34	1.135	p>0.05
	Jul-Aug	78	0.044	p>0.05
	Sep-Oct	59	0.021	p>0.05

2.3.2.4. Length classes

The bimonthly size frequency distribution of mature and unsexed specimens of Drepano and Neochori stations is shown in Figure 2.22. In both stations, the occurrence of four length classes indicated four cohorts per year. The first cohort consisted mainly of unsexed and a few adult individuals with total length (TL) shorter than 80 mm (0+). The second, third and fourth cohorts consisted of adult individuals with total length (TL) shorter than 120 mm (1+), 160 mm (2+), and larger than 190 mm (> 2+) respectively .

In Drepano station all cohorts were present in all of the examined periods, besides the January- February. The second and third cohorts were present in higher numbers than the first and fourth (Figure 2.14). Therefore, adult individuals were present throughout the year and in higher abundance than unsexed. The highest abundance of the first cohort was recorded in the September- October period and the smallest unsexed individuals (less than 50 mm) appeared in the March- April (April), July- August and September- October (October) periods (Figure 2.14). The forth cohort was observed in high abundances in the March- April period (Figure 2.14).

In Neochori station all cohorts were present in all of the examined periods, besides the absence of the first cohort in the January- February period (Figure 2.14). The forth cohort was present in lower abundance than the other three (Figure 2.14). In the March- April period it constituted only of male individuals whereas in the rest of the examined periods it was composed both by males and females (Figure 2.14). High abundance of the first cohort was recorded in the May- June, July- August and September- October periods. The smallest unsexed individuals (less than 50 mm) appeared in the November- December (November) period (Figure 2.14). The second and third cohorts were in high abundances all over the year and they composed both by males and females (Figure 2.14).

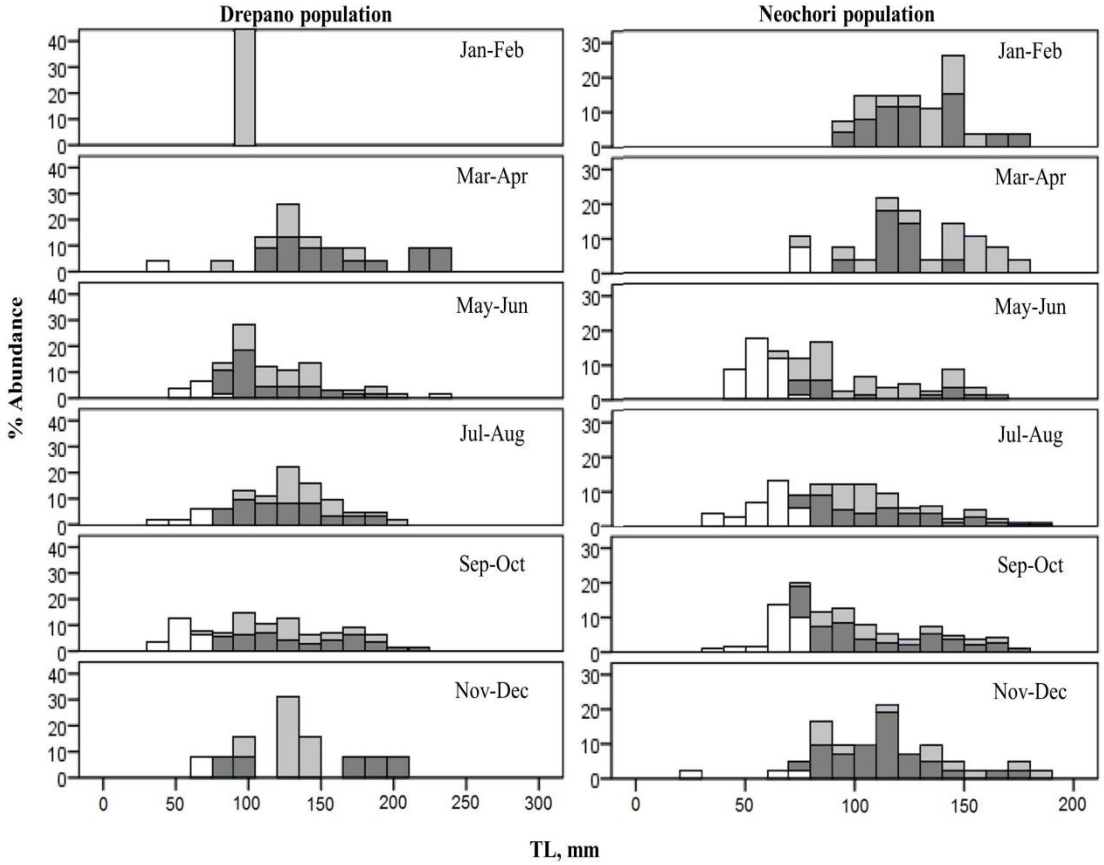

Figure 2 .22. Per cent frequency distributions of total length (mm) of *S. typhle* species caught in Drepano and Neochori stations in the present study (unsexed specimens: white colored; males: light gray colored; Females: dark grey- colored).

Εικόνα 2.22. Ποσοστιαία συχνότητα κατανομής του ολικού μήκους (mm) των ατόμων του είδους *S. typhle* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη παρούσα μελέτη (αδιευκρίνιστου φύλου άτομα: λευκό χρώμα; αρσενικά: ανοιχτό γκρι; Θηλυκά: σκούρο γκρι).

2.3.2.5. Size at maturity (L_{50})

Logistic regression model estimating L_{50} correctly classified 98.7% of males (Hosmer and Lemeshow goodness of fit test: d.f. = 8, $p > 0.05$) and 100% of females (Hosmer and Lemeshow goodness of fit test: d.f. = 2, $p > 0.05$) from Drepano station (Table 2.20). At the same time, males and females from Neochori station were 97% (Hosmer and Lemeshow goodness of fit test: d.f. = 8, $p > 0.05$) and 98.3% (Hosmer and Lemeshow goodness of fit test: d.f. = 6, $p > 0.05$) correctly classified, respectively (Table 2.20).

Lengths at 50% maturity of males and females of *S. typhle* species from Drepano station did not vary (76.1 mm and 75.5 mm respectively) (Figure 2.23). However, the slope of logistic equation was steeper (almost vertical) in females than males. Therefore, females in Drepano station reached maturation length faster than males In Neochori station L_{50} showed that females reached maturation size slightly faster than males (73.7 mm and 75.2 mm respectively) (Figure 2.23). However, the above observed differences were not statistically important.

Table 2.20. Classification results for the cross-validation procedure of logistic regression model estimating L_{50} of male and female specimens of *S. typhle* species caught in Drepano and Neochori stations in the present study.
Πίνακας 2.20. Επανατοποθέτηση των αποτελεσμάτων του μοντέλου λογιστικής παλινδρόμησης για την εκτίμηση του L_{50} των αρσενικών και θηλυκών ατόμων του είδους *S. typhle* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου στην παρούσα μελέτη.

Station	Sex	Recorded sex status	Predicted sex status			Nagelkerke R^2
			Immature	Mature	Percentage	
Drepano	Males	Immature	41	0	100.0	0.96
		Mature	2	111	98.2	
		Overall			98.7	
	Females	Immature	41	0	100.0	1
		Mature	0	132	100.0	
		Overall			100.0	
Neochori	Males	Immature	113	1	99.1	0.933
		Mature	7	144	95.4	
		Overall			97.0	
	Females	Immature	112	2	98.2	0.959
		Mature	3	169	98.3	
		Overall			98.3	

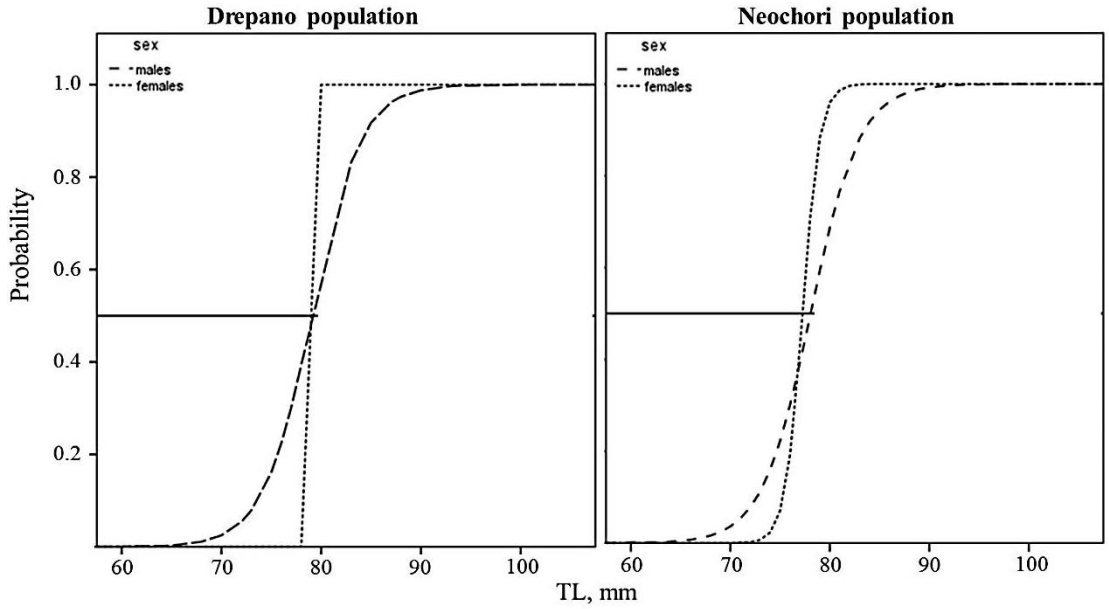

Figure 2.23. Logistic regression curves for the estimation of the L_{50} for male and female individuals, of *S. typhle* species caught in Drepano and Neochori stations during the present study (The interception of the horizontal line and the regression curve correspond to the L_{50}).
Εικόνα 2.23. Καμπύλη λογιστικής παλινδρόμησης για την εκτίμηση του L_{50} των αρσενικών και θηλυκών ατόμων του είδους *S. typhle* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου στην παρούσα μελέτη (Το σημείο τομής της οριζόντιας γραμμής και της σιγμοειδής καμπύλης αντιστοιχεί στο L_{50}).

2.3.2.6. Length-weight relationship (LWR)

The length-weight relationship was estimated for male, female and unsexed individuals of *S. typhle* species during the months with the highest abundances, i.e. reproductive season (May-October) (Table 2.21, Figure 2.24). ANCOVA analysis indicated that all the examined parameters (sex, station, and period) were statistically important in the LWR (Table 2.22). However, the effect of station and its interaction with standard length were marginally significant (p=0.05). The most important parameters that affected the LWR were: i) sex (15.6 %), ii) total length, TL (9.45 %), iii) month (3.7 %) and their interactions iv) (sex * TL: 14.8 %; sex * month * TL: 3.8 %; month * TL: 3.6 %; station * month * TL: 1.9 % and station * sex* TL: 1.1 %).

In both stations males and females had higher b-values than unsexed specimens (Table 2.21, Figure 2.24). Based on the above mentioned observed b- values, males and females in both stations exhibited positive allometric growth pattern, while unsexed specimens isometric pattern (Table 2.21).

Table 2.21. Parameters of the length-weight relationship for male, female and unsexed individuals of *S. typhle* species caught in Drepano and Neochori stations during the reproductive season of the present study (*N*, number of individual; *CI*, confidence interval; +, positive allometric growth pattern; =, isometric growth pattern).

Πίνακας 2.21 Συντελεστές της σχέσης Μήκους- Βάρους των αρσενικών, θηλυκών και αδιευκρίνιστου φύλου ατόμων του είδους *S. typhle* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά την αναπαραγωγική περίοδο της παρούσας μελέτη (*N*, αριθμός των ατόμων; *CI*, διάστημα εμπιστοσύνης; +, θετικά αλλομετρική αύξηση; =, ισομετρική αύξηση).

Station	Sex	Month	N	a		b		R^2	Allometry	
				Value	± 95% CI	Value	± 95% CI		Type	P values
Drepano	Males	May-Jun	32	0.98	0.01	3.60	0.41	0.98	+	<. 0.001
		Jul-Aug	25	0.98	0.01	3.69	0.54	0.97	+	<. 0.001
		Sep-Oct	41	0.98	0.03	3.60	0.21	0.95	+	<. 0.001
	Females	May-Jun	34	0.96	0.01	3.34	0.32	0.97	+	<. 0.001
		Jul-Aug	31	0.95	0.03	3.23	0.19	0.95	+	<. 0.001
		Sep-Oct	47	0.96	0.02	3.34	0.27	0.99	+	<. 0.001
	Unsexed	May-Jun	9	0.77	0.01	2.30	0.53	0.90	-	> 0.05
		Jul-Aug	6	0.86	0.03	2.61	0.26	0.91	-	> 0.05
		Sep-Oct	25	0.93	0.01	3.05	0.38	0.95	=	< 0.05
Neochori	Males	May-Jun	40	0.98	0.03	3.61	0.20	0.95	+	< 0.001
		Jul-Aug	45	0.98	0.03	3.67	0.19	0.95	+	<. 0.001
		Sep-Oct	28	0.98	0.04	3.74	0.16	0.92	+	<. 0.001
	Females	May-Jun	16	0.97	0.03	3.47	0.21	0.97	+	< 0.05
		Jul-Aug	50	0.97	0.04	3.48	0.16	0.96	+	<. 0.001
		Sep-Oct	52	0.97	0.06	3.50	0.11	0.98	+	<. 0.05
	Unsexed	May-Jun	36	0.91	0.01	2.91	0.41	0.98	=	<. 0.001
		Jul-Aug	42	0.91	0.03	2.90	0.23	0.97	=	<. 0.001
		Sep-Oct	31	0.91	0.04	2.91	0.17	0.98	=	<. 0.05

Table 2.22. Factors affecting the length- weight relationship of *S. typhle* species from Drepano and Neochori populations as shown by the results of the ANCOVA analysis in the present study (*F*, values of significance test; *P-values*, level of significance *, interaction).
Πίνακας 2.22. Παράγοντες που επηρεάζουν τη Σχέση Μήκους-Βάρους του είδους *S. typhle* σύμφωνα με τα αποτελέσματα της Ανάλυσης Διακύμανσης, από δείγματα που συλλέχτηκαν στους σταθμούς το Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της παρούσας μελέτης (*F*, τιμές ελέγχου σημαντικότητας; *P-values*, επίπεδο σημαντικότητας, * αλληλεπίδραση).

Factor	**F**	**P- values**	**Partial Eta Squared**	R^2
TL	15082.62	0	0.945	
Station	3.848	0.05	0.004	
Sex	80.539	0	0.156	
Month	5.55	0	0.037	
Station * TL	3.836	0.05	0.004	
Sex * TL	75.388	0	0.148	0.963
Month * TL	5.463	0	0.036	
Station * Sex * TL	4.798	0.008	0.011	
Station * Month * TL	2.758	0.012	0.019	
Sex * Month * TL	2.839	0.001	0.038	

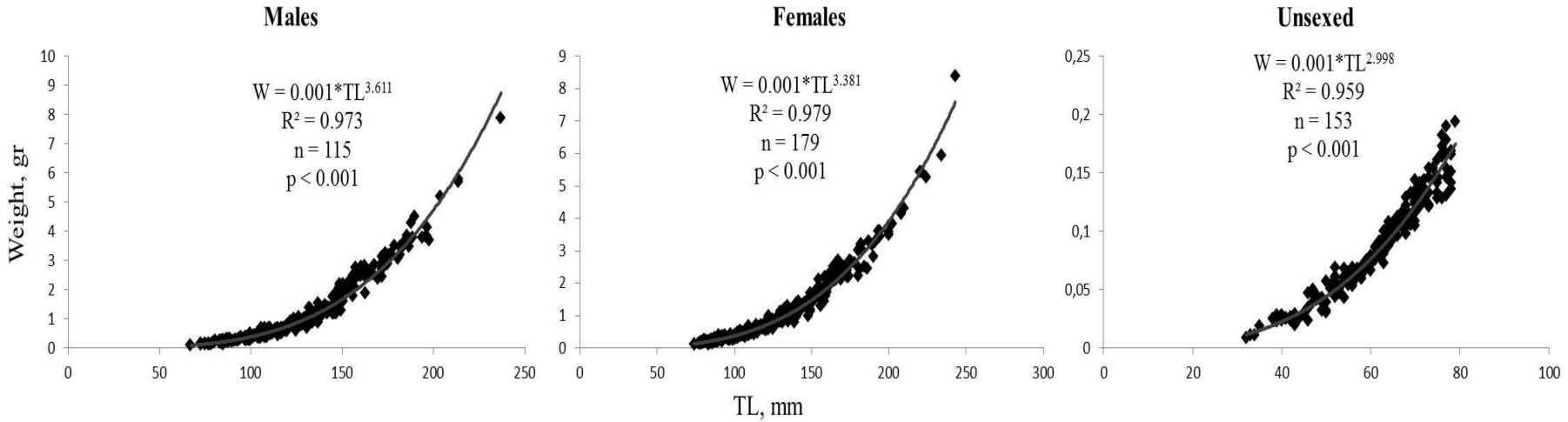

Figure 2.24. . Total length-total weight relationship (mm, gr) of male, female and unsexed individuals of *S. typhle* species caught in Drepano and Neochori stations during the reproductive period of the present study.

Εικόνα 2.24. Σχέση ολικού μήκους-ολικού βάρους (mm, gr) αρσενικών, θηλυκών και αδιευκρίνιστου φύλου ατόμων του είδους *S. typhle* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της αναπαραγωγικής περιόδου της παρούσας μελέτης.

2.3.2.7. Gonadosomatic Index (GSI)

Values of gonadosomatic index were higher for females than males in both stations (Figure 2.25). In Drepano station, GSI of females peaked in March-April period (March) while of males' peaked in January-February (end of February) period. The lowest values for males were recorded in the July-August and for females in the September-October periods. The variance of GSI of males was lower than females (Figure 2.25).

In Neochori station, the periods with the higher GSI values for females were March-April and July-August while the periods with the lowest values were November-December. Males' index reached peak in May-June month and low in January-February. As in the case of *S. abaster* species, the GSI pattern of the Neochori station was antisymetrical i.e. the highest values for females were recorded when the lowest values were observed for males and vice versa (Figure 2.25).

ANCOVA analysis showed that sex (31.9%), month (9.0 %), the interaction of month and sex (7.5%), station and month (0.4%) and the interaction of sex, month and station were the statistically significant factors that influenced the GSI. The station-parameter was not significant (Table 2.23).

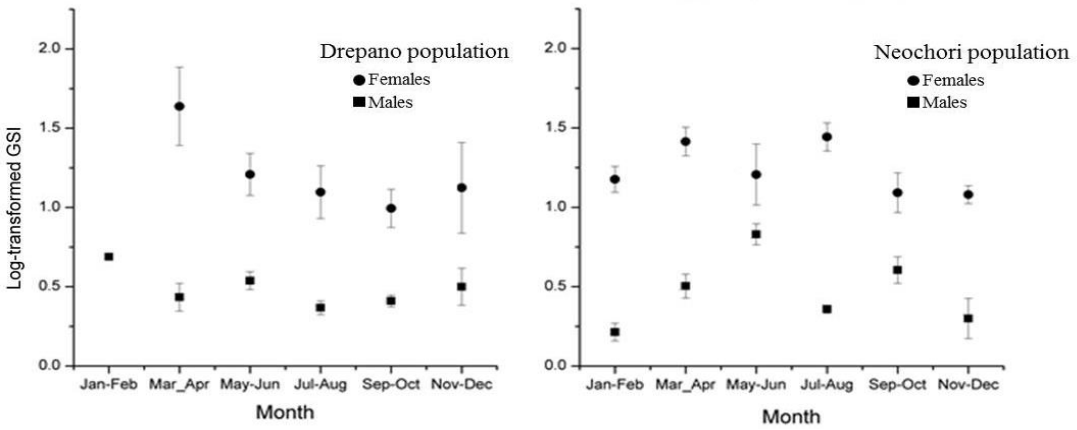

Figure 2.25. Error bars of the variation of gonadosomatic index (GSI) for male and female individuals of *S. typhle* species caught in Drepano and Neochori stations during the present study (T-bars that extend from the points correspond to the standard deviation).

Εικόνα 2.25. Διαγράμματα σφαλμάτων της διακύμανσης του γοναδοσωματικού δείκτη (ΓΣΔ) των αρσενικών και θηλυκών ατόμων του είδους *S. typhle*, τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη παρούσα μελέτη (οι γραμμές σχήματος «Τ» αντιστοιχούν στην τυπική απόκλιση του δείγματος).

Table 2.23. Factors affecting the values of gonadosomatic index (GSI) of *S. typhle* species as shown by the results of the ANCOVA analysis. The specimens used in the present study were caught in Drepano and Neochori stations (*F*, values of significance test; *P-values*, level of significance *, interaction).
Πίνακας 2.23. Παράγοντες που επηρεάζουν τον γοναδοσωματικό δείκτη (ΓΣΔ) του είδους *S. typhle* σύμφωνα με τα αποτελέσματα της Ανάλυσης Συνδιακύμανσης. Τα άτομα που χρησιμοποιήθηκαν στη παρούσα μελέτης συλλέχθηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου (*F*, τιμές ελέγχου σημαντικότητας; *P-values*, επίπεδο σημαντικότητας, * αλληλεπίδραση).

Factor	F	P-values	Partial Eta Squared
Sex	216.771	0	0.319
Month	4.169	0	0.09
Month * Sex	3.77	0	0.075
Station * Month * Sex	4.831	0	0.05
Station * Month	3.153	0.003	0.045
Station * Sex	1.984	0.16	0.004
Station	0.353	0.553	0.001

2.3.2.8. Hepatosomatic Index (HSI)

Values of hepatosomatic index, generally, were higher in females than males in both stations (Figure 2.26). In Drepano station, the values of HSI of females and males peaked in the November-December period. The lowest value for females was recorded in the September-October while for males in the March-April period (Figure 2.26).

In Neochori station, the highest value of HSI for females was recorded in the March-April period while the lowest in the September-October. Males' index reached peak in the January-February period and low in May-June. The values of HSI displayed restricted variance in both sexes (Figure 2.26).

ANCOVA analysis revealed that sex (31.9%), month (9%) and the interactions of sex, moth and station were the statistically significant factors that influenced the HSI. On the other hand, the station and its interaction with sex were not significant factors affecting the hepatosomatic index (Table 2.24).

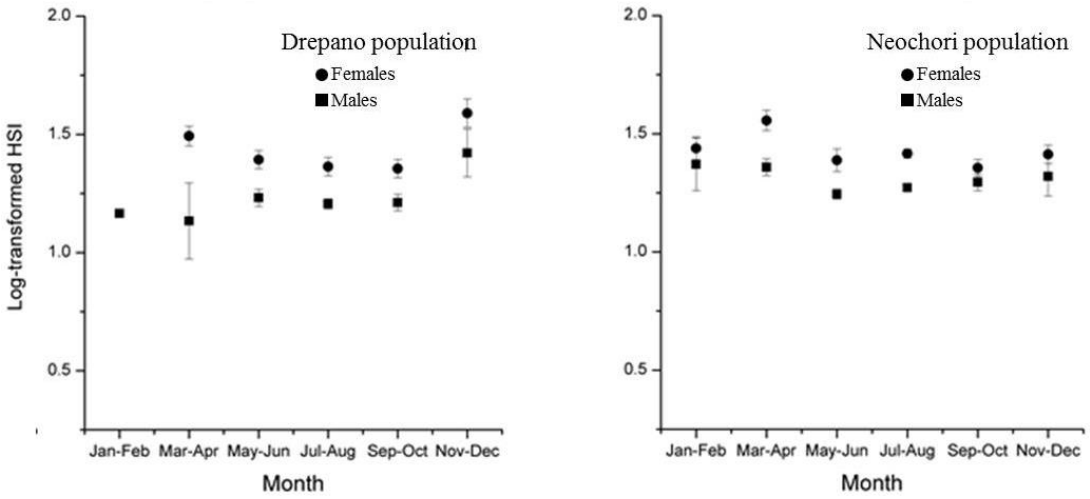

Figure 2.26. . Error bars of the variation of hepatosomatic index (HSI) for male and female individuals of *S. typhle* species caught in Drepano and Neochori stations during the present study (T-bars that extend from the points correspond to the standard deviation).
Εικόνα 2.26. Διάγραμματα σφαλμάτων της διακύμανσης του ηπατοσωματικού δείκτη (ΗΣΔ) των αρσενικών θηλυκών ατόμων του είδους *S. typhle* τα οποία συλλέχτηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη παρούσα μελέτη (οι γραμμές σχήματος «Τ» αντιστοιχούν στην τυπική απόκλιση του δείγματος).

Table 2.24. . Factors affecting the values of hepatosomatic index (LSI) of *S. typhle* as shown by the results of the ANCOVA analysis. The specimens used in the present study were caught in Drepano and Neochori stations (*F*, values of significance test; *P-values*, level of significance *, interaction).
Πίνακας 2.24. Παράγοντες που επηρεάζουν τον ηπατοσωματικό δείκτη (ΗΣΔ) του είδους *S. typhle* σύμφωνα με τα αποτελέσματα της Ανάλυσης Συνδιακύμανσης. Τα άτομα που χρησιμοποιήθηκαν στη παρούσα μελέτης συλλέχθηκαν στους σταθμούς του Δρεπάνου και του Νεοχωρίου (*F*, τιμές ελέγχου σημαντικότητας; *P-values*, επίπεδο σημαντικότητας, * αλληλεπίδραση).

Factor	F	P-values	Partial Eta Squared
Sex	216.771	0	0.319
Month	4.169	0	0.09
Month * Sex	3.77	0	0.075
Station * Month * Sex	4.831	0	0.05
Station * Month	3.153	0.003	0.045
Station * Sex	1.984	0.16	0.004
Station	0.353	0.553	0.001

2.4. DISCUSSION

The results of the analysis suggest the existence of established breeding populations of *S. abaster* and *S. typhle* species in Drepano and Neochori stations with no evidence of interspecific competition. The examined biological features of the two species in Drepano and Neochori station are discussed in the following pages.

2.4.1. *Syngnathus abaster* species

S. abaster individuals were present all year around in Drepano and Neochori stations in relatively equal numbers (Figure 2.13). As already discussed the dominant vegetation types in Drepano and Neochori station are *Cymodocea nodosa* (sparse and patchy seagrass beds) and *Posidonia oceanica* (dense vegetation) respectively (Materials and Methods part of the present chapter). Malavasi et al. (2007) showed that *S. abaster* species in Venice lagoon prefer sites dominated by dense eelgrass while Kendrick and Hyndes (2005) indicated that syngnathids prefer dense vegetation than sparse one. The lack of deviation in the species abundance in two ecosystems of different type and density of vegetation indicates that the species abundance in the N. Ionian Sea was regulated by other environmental and abiotic factors than vegetation. Thus, the appropriate habitats for these fishes may not be determined simply by the presence or type of vegetation.

As shown, the species abundance differed during the study in both stations (Figure 2.11). More specifically, during winter months when the temperature was low, the species numbers were in low levels too (despite the fact that data from two consecutive years were merged). As water temperature increased from winter lows to spring, more individuals appeared in the collection sites, climaxing during late summer and early autumn period. This fluctuation in the species abundance indicates that during winter months (when a huge part of the seagrass died, water temperature fell, food became spare and environmental conditions became harsh i.e. strong wave activity, currents and turbidity) the populations migrated to more protected areas (deeper, warmer waters outside of the sampling range of the present study) for overwintering. During spring, summer and early autumn months (when the seagrass community revived, water temperature rose and environmental conditions became milder) the sublittoral zone provided, again, a sheltered environment with high food supplies for adult and juvenile specimens. Seasonal migration is a common strategy among temperate syngnathids (e.g. Lazzari and Able 1990; Cambel and Able 1998; Bolland and Boettcher 2005; Ripley and Foran 2006) and has also been observed in populations of *S. abaster* in the Adriatic Sea (Franzoi et al 1993, Riccato et al. 2003). However, against the general pattern some individuals were found in the sublittoral zone in winter months. This could either be a random event (e.g. some individuals left behind or got carried in the seashore by the waves) or could be an indication that some adult individuals of *S. abaster* remain within the sublittoral zone year-round. Such an exception was also observed in *Hippocampus guttulatus* population in Ria Formosa lagoon (Portugal) (Curtis and Vincent 2006)

S. abaster consisted of two length classes in both stations, indicating that the species lifespan exceeded 12 months and was probably around 18-20 months, similarly to the Adriatic Sea population (Franzoi et al. 1993). During winter months there was not recorded a significant change in length of unsexed individuals compared to the rest of the sampling periods. This fact could be attributed to the prolonged reproductive period and/or to a slow growth rate during the overwintering period in deeper waters. A lack of growth during winter months is common for many other temperate fishes (Szedlmayer et al. 1992; Keefe and Able 1993). This lack of growth and the reduction in the number of smaller individuals (< 70 mm TL) from fall to spring suggested overwinter mortality, which has also been reported for *S. abaster* species in the Venice lagoon (Franzoi et al. 1993) but also for *Syngnathus scovelli* (Campbell and Able 1998).

Among adult individuals both sexes in both stations displayed a positive allometric growth pattern. An allometric growth pattern was also, observed in the population of Strymon Estuaries (N. Aegean Sea) (Koutrakis and Tsikliras 2003). The steep (almost vertical in some cases) slopes in the logistic equations for the estimation of the L_{50} in both stations could be explained in the context of an opportunistic or intermediate life strategy (King and. McFarlane 2003). In particular *S. abaster* as a short-lived species has cannot afford to delay maturation. Thus, most of its specimens mature within the first year in a relatively similar size (Franzoi et al. 1993; King and. McFarlane 2003). However, in order to characterize a species as opportunistic or intermediate strategist more data on its growth rate, maximum size and fecundity are needed (King and. McFarlane 2003).

S. abaster individuals matured and were ready to reproduce when they reached approximately 70 mm. Reproduction period started in early spring (March or April) and lasted until early October (the reasons supporting the start and the end of the reproductive period are discussed below). The same duration was also recorded in *S. abaster* populations from the Adriatic Sea and the Ria Fermossa (Portugal) (Riccato et al. 2003; Silva et al. 2006a) but also in populations of *Syngnathus acus* from the S. Aegean Sea (Gurkan et al. 2009). Compared to the Central and N. European syngnathid populations (Berglund 1991; Vincent et al. 1994; 1995), the reproductive period of *S. abaster* in the N. Ionian Sea is almost twice as long. Water temperature is known to be the predominant factor controlling both the starting of the reproductive period and the breeding cycle of syngnathids (e.g. Berglund 1991; Bolland and Boeticher 2005; Silva et al. 2007; 2010). The temperatures recorded in the present study are higher than those recorded in the studies of Berglund (1991) and Vincent et al. (1994; 1995) Therefore, the high water temperature could be a reason for the extended reproductive period in the N. Ionian Sea populations of *S. abaster* species. However, this conclusion needs to be tested with contemporary data on the populations of the N.C. Mediterranean and European coastline

The beginning of the reproductive season was evident by the following factors:

1. Females GSI peaked in March indicating that females had formed ripped ovaries and were ready to reproduce. In the same period, males' index rose also, indicating that brood pouches were ready to accept eggs.
2. In females, increase in HSI values corresponded to increased production of proteins associated with the development of the ovaries; vitellogenin (an egg yolk precursor protein) (Henderson et al. 1996; Gutjahr-Gobell et al. 2002). Also HSI is an estimator of the energy status of the fish (Wootton et al. 1978; Campbell and Love 1978). Therefore, the high values of HSI for both sexes in the March-April (March) period indicated that females and males were ready for the energy demanding process of reproduction and male pregnancy (osmoregulation, nutrition and protection of the eggs and the embryos).
3. During the late autumn and winter months (November-February), males brood pouch was undeveloped. At the beginning of spring (March) the pouch was shaped but in the captured males was still empty. Brooding males were captured in the May-June (May) period. Given that male pregnancy lasts a little more than one month, these brooding males must have copulated in late March or early April.
4. In Drepano station unsexed specimens were present all year long even in the January- February period. However, in the beginning of the spring their total length (28 mm) was smaller than winter's (58 mm). Silva et al. (2006a) showed that the newborn juveniles of *S. abaster* are approximately 20 mm long. The length of the unsexed individuals combined with the absence of brood pouch in the winter months of the present study, indicated that the unsexed specimens collected in the March- April period (April) were the first young of that year's reproductive period (YOY). Therefore, these juveniles must have been conceived in the beginning of March, signaling the beginning of the reproductive period. The unsexed specimens observed during winter months were juveniles born in last year's reproductive period and had not yet matured.
5. In Neochori station unsexed specimens were present from March-April period to November-December. For the same reasons explained for Drepano station the first YOY were recorded in the May-June period (beginning of May) and thus, must have been conceived in the beginning of April or the end of March.

During the reproductive period the operational sex ratio of both populations was female biased. This outcome was expected as female biased operational sex ratio is an indicator of sex role reversal (Clutton- Brock and Vincent 1991). All of the European populations of *S. abaster* studied so far are female biased (Silva et al. 2006b; Cunha 2012; Hubner et al. 2013) and the populations from Drepano and Neochori were not an exception. The absence of length sexual dimorphism was also expected as *S. abaster* is a "mild" sex-reversed species, whose populations show moderate signs of sexual selection (Silva et al. 2006b).

However, an unexpected outcome was the absence of length difference between brooding and non-brooding males. This fact is incompatible with the reproductive pattern of *S. abaster* and European pipefishes in the so far studied populations i.e. in the beginning of the reproductive period, both sexes prefer larger partners while smaller males and females are neglected (Berglund et al. 1986, Berglund and Rosenqvist 1993, Silva et al. 2009). However, in the population of N. Ionian Sea that pattern was observed. Temperature is one of the main factors affecting sexual selection (Kvarnemo 1996; Kvarnemo and Ahnesjo 1996; Andersson and Iwasa 1996). The prolonged reproductive period (due to the high temperatures) observed in the N. Ionian Sea most possible provided both small and large males the opportunity to mate at any point of the reproductive cycle, without any hurry. This possible absence of time pressure for both males and females could have led to the lack of a size-guided reproduction pattern throughout the reproductive period (Mobley et al. 2013).

Furthermore, gonadosomatic index differed between the individuals of the two stations. More specifically GSI of both sexes in Drepano station peaked in March remained in high levels during summer months and then fell. In Neochori station, females and males index peaked twice. For females, the first peak was recorded in the begging of the spring (March) and the second in the summer (July and August) period. For males the two peaks occurred in the end of Spring and the beginning of autumn. A possible reason for that difference could be the synchronization –or not- of male pregnancy (Vincent et al. 1994; Sogabe and Yanagisawa 2007). Specifically, in the beginning of the reproductive period, specimens arrived in the shore to breed (Vincent et al. 1994). Almost all individuals of both sexes were ready to mate simultaneously, justifying the high values of males and females GSI. The males that filled their brood pouch consisted the first batch of brooding males and were no longer available to mate. The remaining males would keep trying to reproduce until they succeeded, consisting the second batch of brooding males and so on. Males that mated during the same time would give birth almost synchronously, too. No matter if males mated in the very beginning of the reproductive season or were a second or a latter batch, once the embryos were released they were available to reproduce again as females gonads were still rip- as suggested by the high values of GSI.

As already mentioned the GSI of males increases when they are ready to accept a brood, falls during pregnancy and increase again before the release of the embryos and the new copulation (Mayer 1993; Kornienko 2001; Bolland and Boettcher 2005). The fact that, in Neochori station the GSI of males and females followed a distinct fluctuation pattern after the first reproductive activity indicates that most males released their embryos, and mated again pretty much at the same period; synchronization of pregnancy. On the contrary, in Drepano station the evenness of males and females GSI after the first two months of the reproductive period indicates the existence of many male brooding batches; unsychronization of pregnancy. The phenomenon of synchronized male pregnancy was also observed in the population of *S. abaster* from Adriatic Sea (Riccato et al. 2003) but also in the Swedish population of *S. typhle* species (Vincent et al. 1994).

The first YOY recruit occurred in the March-April and in the May-June periods in Drepano and Neochori stations, respectively. The following months the continued range and swift in the histograms frequency of the juveniles' total length indicated mainly a growth of the YOY individuals rather than new recruits. In the November-December period the specimens of Drepano station with total length around 50 mm were juveniles and not newborns while those from Neochori were newborns (Silva et al. (2006a) showed that newborns total length is approximately 20 mm). Therefore, there was only one major YOY recruit in Drepano station but two in Neochori. The difference in the YOY was probably correlated to the above mentioned synchronization or not of male pregnancy. The low numbers of newborn individuals (total length approximately 20 mm) in both stations could also depict an inadequacy of the mesh size (2 mm) in the beach seine and the hand net to capture so small sized specimens.

Finally, the reproductive period ended in both stations in late September or early October. This was indicated by the fact that even though pregnant males were recorded in both stations in October they were absent in November. In the latter month only juveniles were present which could have been conceived in the end of the reproductive period. Another indication for the end of the reproductive season was the low values of GSI in September and October months. Females GSI dropped, indicating that individuals with ripe ovaries were rare (e.g. Rajasilta et al. 1997; Sadekarpawar and Parikh 2013). The rise in the GSI values of males in Neochori station could indicated that they were ready to accept new batch of eggs if females had, still, ripe ovaries and/or if environmental conditions were favorable (e.g. Rajasilta et al. 1997; Sadekarpawar and Parikh 2013). However examination of the gonad is necessary before reaching any final conclusions. Such a prolonged reproductive period was also observed in species populations from the Adriatic Sea and the Ria Fermossa (Portugal) (Riccato et al. 2003; Silva et al. 2006a) most probably due to the similar ambient temperatures prevailing in these areas during that period (direct comparison of the temperatures mentioned in the present thesis and the other two studies).

2.4.2. *Syngnathus typhle* species

S. typhle specimens were present in Drepano and Neochori stations all year long without a statistical significant difference in their abundance (Figure 2.19). This outcome contradicts the results of Malavasi et al. (2007) on the species preference on longer leaves and intermediate shoot density (*Cymodocea nodosa* in Drepano station) over shorter and more dense seagrass leaves (*Posidonia oceanica* in Neochori station). This lack of preference –similar to *S. abaster* species- is yet one more indication that habitat preference is a multidisciplinary process influenced by a combination of factors, such as structural complexity, predation and competition and is not dictated only by one factor such as type or coverage of vegetation (Schofield 2003, Warfe and Barmuta 2004).

The abundance of *S. typhle* in the both stations varied during the present study. A seasonal migration pattern is most possibly the cause for this variation as in the case of *S. abaster*. According to this scenario *S. typhle* specimens spend winter months in deeper and warmer water and return to the sublittoral zone from spring until autumn, when temperature rises again and environmental conditions are more favorable. This migration pattern is also in accordance with the so far studied populations of *S. typhle* from the Adriatic and Baltic Sea (Franzoi et al. 1993; Riccato et al. 2003; Miersch 2012). Despite the norm of seasonal migration, it is indicated that a small number of individuals may overwinter in sublittoral seagrass beds (Dawson 1986), accounting for the specimens caught during winter months mainly in Neochori station.

The length frequency distribution of *S. typhle* in both stations indicated that the species life span consisted of two and possible up to four age classes. This outcome succeeds the so far mentioned lifespan in the Swedish and the Adriatic Sea populations (Vincent et al 1995; Franzoi et al. 1993; Riccato et al. 2003). During winter months there was not recorded a significant change in length of unsexed individuals compared to the rest of the sampling periods. This fact could be attributed to a long reproductive period and/or to a slow growth rate during the overwintering period in deeper waters, similarly to *S. abaster* populations of the present study (Szedlmayer et al. 1992; Keefe and Able 1993). Also the reduction in the number of smaller individuals, in combination with the slow growth rate during the colder months indicated the effect of overwinter mortality, similarly to the species Swedish and Adriatic Sea population (Vincent et al. 1994; Franzoi et al. 1993) and other syngnathids (Franzoi et al. 1993; Campbell and Able 1998).

Among adult individuals, both males and females displayed a positive allometric growth pattern in both stations. The so far existing data on populations from the Aegean Sea and W. Mediterranean were conflicting, revealing an isometric and positive allometric growth pattern respectively (Gurkan and Taskavak 2007; Valle et al. 2003). The length ranges in both studies overlapped with the N. Ionian Sea population so the results were comparable. More specifically, the results of the present study agree with the growth pattern observed in the W. Mediterranean Sea. The length-weight relationship in fishes can be affected by physiological (e.g. sex, stomach fullness, gonad maturity) and abiotic

factors (e.g. season, habitat) (Tesh 1971; Wootton 1998). However most of these data were not available in the study of Gurkan and Taskavak (2007) and therefore the reason for the discrepancy in the growth rate pattern could not be further investigated.

The logistic equations for the estimation of the L_{50} in both stations revealed a steep slope for females and an almost vertical slope for males indicating a synchronized maturation of both sexes at similar lengths. Similarly to *S. abaster* species, this pattern could be explained in the context of an opportunistic or intermediate life strategy (King and. McFarlane 2003). A similar pattern was also observed in the species population from Baltic Sea in order to maximize the reproductive success of both sexes (Vincent et al. 1994).

S. typhle individuals in N. Ionian Sea matured when they reached about 70 mm. This size at maturity is considerably smaller compared to the Swedish and the Adriatic Sea populations (Vincent et al. 1994; Riccato et al. 2003). Reproductive period started in early spring and lasted until October. The same period was also recorded in species populations from the Venice Lagoon (Adriatic Sea) (Riccato et al. 2003), the Ria Fermosa Lagoon (Portugal) (Freire 2004) and the Gulf of Biscay (Dunker 1908 according to Rispoli and Wilson 2008). The breeding period of northern populations is substantially shorter lasting from May until the end of August (Berglund et al. 1986; Duncker 1908 according to Rispoli and Wilson 2008). As already mentioned, the duration of the reproductive period is positively correlated to temperature rise (reviewed by Berrigan and Charnov 1994). Therefore, the result of the present study is one more indication that the reproductive period of southern syngnathids -that inhabit warmer waters- is longer than the northern populations which inhabit colder water (e.g. Berglund 1991; Vincent et al. 1994; Bolland and Boeticher 2005; Wilson et al. 2008).

The beginning of the reproductive period was evident by the following factors-similarly to its congeneric *S. abaster* species:

1. During the late autumn and winter months (November-February), the male brood pouch was undeveloped. In the beginning of the spring (March-April period) the pouch was shaped and full in more than 50% of the captured males. Given that, male pregnancy in warm waters lasts a little more than one month (Rispoli and Wilson 2008), captured brooding males must have copulated in the beginning of March.
2. Females GSI peaked in early spring, indicating that females had formed rip ovaries and were ready to reproduce. In the same period, males' index was in low levels indicating that their gonads were not full, even though they had shaped brood pouches. Thus, some males had already mated and inseminated their eggs.
3. In females, increased values of HSI during that period could probably correspond to the production of proteins associated with the development of the ovaries e.g. vitellogenin (an egg yolk precursor protein) (Henderson et al. 1996, Gutjahr-Gobell et al. 2002). Also HSI is an estimator of the energy status of the fish (Wootton

1984). Therefore, both sexes in the beginning of the spring were ready for the energy consuming proses of reproduction and male pregnancy.

4. Unsexed specimens were present all year long except from winter months. The first unsexed individuals appeared in both stations in the April. In Drepano station these individuals were shorter than 4 mm while in Neochori station they were longer than 5 mm. Given that the mean total length of the species newborn individuals is 26.2 mm (Chapter 5 of the present thesis) only the individuals in Drepano station were first YOY (conceived in the beginning of the period) of the reproductive period. Therefore, they must have been conceived in the begging of March, signaling the onset of the reproductive period. Contrary in Neochori station these were juveniles born in the previous reproductive year which had not yet matured.

During the reproductive period the operational sex ratio in both stations was female biased. This outcome was expected as the species is sex- role reversed and female biased operational sex ratio is an important indicator of this behavior (Clutton- Brock and Vincent 1991). The so far studied population of *S. typhle* revealed unbiased or female biased populations (Vincent et al. 1995; Franzoi et al. 1993; Riccato et al. 2003; Oliveira et al. 2007). However, in Neochori station and only in May the OSR was male-biased. This short-term bias of the OSR observed in the first months of the reproductive period was due to the fact that the number of available males was clearly greater than the number of available females. As males' pouches filled, the OSR was reversed and female biased once again. Therefore, this single event cannot be considered as an indicator of male competition and sex role reversal. The so far studied population of *S. typhle* revealed unbiased or female biased populations (Vincent et al. 1995; Franzoi et al. 1993; Riccato et al. 2003; Oliveira et al. 2007). Similar temporal swifts in the OSR during the onset of the reproduction season were also observed in the species Swedish population (Vincent et al. 1994; 1995).

An interesting fact observed during the reproductive period was the statistically important difference in the total length of brooding and non-brooding males which was not noted in the studied population of *S. abaster*. In both stations, brooding males were larger than non-brooding in the end of the reproductive period, while only in the Neochori station brooding males were also larger in the beginning of the reproductive period. However, during the same period, no difference was observed in the total length of female individuals in neither station. The difference in the non-brooding and brooding males could mean that during the beginning or end of the breeding period: i) the larger-sized individuals were the first or the last to arrive or stay respectively in the stations and thus the only available males for mating or ii) despite the presence of smaller sized individuals during that period, females preferred larger mates. The first hypothesis is rejected as the length frequency distribution in both stations showed a wide range of body lengths during these specific periods; both smaller and larger sized males were present in the population. On the other hand, *S. typhle* is a polygynandrous species (Jones and Avise 2001). As

already mentioned, in polygynandrous species the intensity of sexual selection is weak or variable (Avise et al. 2002). This means that, either sex could be the choosy one depending on the circumstances. It is known that *S. typhle* species prefer larger partners when mating while smaller males and females are neglected (Berglund et al. 1986; Berglund and Rosenqvist 1993). Therefore, the length difference between larger brooding and smaller non-brooding males could be attributed to females' preference for larger-sized partner. Why this preference existed only in these bimonths or why there is no male preference on larger females are questions that cannot be answered by the present study's data.

Furthermore, the pattern of the GSI values differed between the two populations. More specifically, in Drepano station females GSI peaked in March-April period and then was retained in lower but similar levels for the rest of the examined period. Males GSI fluctuation was moderate. In Neochori population, GSI of both sexes fluctuated with a more intense pattern than Drepano's population. In particular, females GSI rose in the begning and the middle of the reproductive period. Males GSI followed a reverse pattern i.e. fell in March-April, and July-August periods and rose in May-June and September-October. As already mentioned, similar pattern in the fluctuation of the GSI values in each staion occurred in the sympatric *S. abaster* species as well as in Swedish and Adiatic Sea populations of *S. typhle* (Vincent et al. 1994; Riccato et al. 2003) . Therefore, it could be deduced that Drepano population breed in continuous batches throughout the reproductive period –unsynchronized male pregnancy-, while in Neochori population two and possibly three major brooding batches occurred- synchronized male pregnancy (Mayer 1993; Kornienko 2001; Bolland and Boettcher 2005).

Finally, the reproductive period ended in late September or early October similarly to *S. abaster* populations. This was obvious by the fact that even though pregnant males in both stations were recorded in October they were not present in November. Furthermore, in females of both stations the values of GSI dropped in the September- November period indicating that individuals with ripe ovaries were rare (e.g. Rajasilta et al. 1997; Sadekarpawar and Parikh 2013). As already mentioned a similar reproductive period was also recorded in population in the Adriatic Sea (Riccato et al. 2003), the Ria Fermosa Lagoon (Freire 2004) and the Gulf of Biscay (Dunker 1908 according to Rispoli and Wilson 2008).

2.4.3. Male pregnancy synchronization

The study of the biological cycle of *S. abaster* and *S. typhle* revealed differences and resemblances between the individuals in Drepano and Neochori stations. However, there is a difference that seems to follow a station specific pattern in both species; the GSI values. As already mentioned males' GSI exhibited an intense fluctuation in Neochori station while a more moderate one in Drepano. This difference is most possible attributed to the synchronization of male pregnancy (Vincent et al. 1994).

Synchronized spawning periods have been recorded for marine and freshwater teleost fish species (e.g. Cushing 1969; Brown and Scott 1994), aiming to ensure high chances of survival for the offspring (e.g. Verhulst et al. 1995; Van Der Kraak and Pankhurst 1997). Photoperiod and temperature are the main factors synchronizing reproduction in temperate species by controlling the synthesis of gametogenesis- related proteins such as vitelogenenin (Johannes 1978; Henderson et al. 1996; Gutjahr-Gobell et al. 2002). Besides these two main factors, synchronization of reproduction may play an important role in increasing survival against predation (Johannes 1978; Hatchwell 1991) and reproductive success (Rowe and Hutchings 2003). Also synchronization according to lunar cycles is commonly viewed mainly in intertidal species (e.g. Takemura et al. 2004, Celik and Celik 2011).

As already mentioned, Neochori station provides a protected environments from the wind and waves. Also, the dense and long leaves of *Posidonia oceanica* provides pipefishes with a more secure and sheltered environment against predators (Vincent et al. 1995; Malavasi et al. 2007). Therefore, male pregnancy could be synchronized as the possibility of all pregnant males to lose their offspring the same period due to predation is relatively low (Johannes 1978; Hatchwell 1991). Contrary, in Drepano station the open sea broad fetch and the short and less dense leaves of *Cymodocea nodosa* leave pipefishes exposed to harsh environmental conditions and predation. These conditions can cause disruption of the reproductive activity and unfavorable conditions for the juvenile release. Thus, Drepano populations could have adopted unsynchronized male pregnancy pattern to avoid jeopardizing all their offspring at once. This could probably indicate that the reproductive timing is not a species specific pattern but rather a habitat related one with intrinsic factors affecting when an individual- and a population in a boarder sense- chooses to spawn (Cushing 1969; Brown and Scott 1994; Vincent et al. 1995 Van Der Kraak and Pankhurst 1997).

Chapter 3. Genetic structure of *Syngnathus abaster* and *Syngnathus typhle* species along the Greek coastline

3.1. INTRODUCTION

3.1.1. Evolutionary markers

Evolution is defined as the variation of the hereditary characteristics of all forms of life over consecutive generations (Ridley 1993). As these differences accumulate (through mutations, genetic drift and natural selection) they could lead to differentiation at the level of population, species and higher order taxonomic groups (Linda and Paul 1995; Avise et al. 2002; Chauhan and Rajiv 2010).

The advent of molecular markers in the midst of the 20th century- and their progress in the following decades- has transformed the fields of evolution and ecology as provided a powerful tool to detect genetic variation at all taxonomical levels, reconstruct the phylogenetic relationships of many taxa and solve problems related to parentage analysis (Parker et al. 1998; Jones and Ardren 2003; Avise 2004; Chauhan and Rajiv 2010; Mobley et al. 2011). Molecular markers are specific fragments of the DNA that are representative of genome differences and can or cannot be associated with the phenotypic expression of a trait (Avise 1994; Agarwal et al. 2008). They can either be protein (allozymes) or DNA (mitochondrial and nuclear) markers (Avise 1994).

Allozymes were the first molecular markers to be used widely to explore the phylogenetic relationships and the genetic structure of populations (e.g. Avise 1994; Bembo et al. 1996; Mamouris et al. 1998; Chauhan and Rajiv 2010). They are different allelic forms of the same locus that are highly preserved throughout the higher taxonomic classes (Avise 1994; Parker et al. 1998). The major advantages of these codominant markers are i) their low cost and ii) the effectiveness to detect levels of intrapopulation variation (Avise 1994). However, allozymes have lost ground to DNA markers as they i) are sensitive to both the quantity and the quality of the used tissue, ii) cannot detect silent and synonymous substitutions and iii) can sometimes be instable (Avise 1994).

In most of the studies that use molecular markers, the predominant classes are mitochondrial DNA (for population evolution and genealogy studies) and microsatellite nuclear DNA (to study the interaction between the dynamics of population and their genetic structure). The mitochondrial genome is typically compact at 16 kb with few intergenic spacers, containing 37 genes (responsible for 13 proteins), 2 rRNAs and 22 tRNAs encoded in the light (L) and the heavy (H) DNA strand (Meyer 1993). mtDNA is a suitable marker for phylogenetic research as it: i) is inherited through the maternal line, constituting thus a clonally inherited marker for maternal lineages and ii) has a high level of variability and mutational rate, even though it is not encoding many proteins. The most polymorphic region of the mtDNA is the control region (D-loop) (Brown et al., 1979) and therefore it is widely used in phylogenetic studies (Gillham 1994, Rokas et al. 2003). Due to the fact that the mitochondria exist in many copies per cell, they are relatively easy to isolate and use in molecular protocols (high recovery probability).

Over the past decades several regions of the mitochondrial genome were used in phylogenetic studies of several taxa, from family to population level. The major

disadvantage of this marker is the existence of mitochondrial pseudogenes inside the genome of many organisms (Zhang and Hewitt 1996a). These types of sequences are numerous in many organisms and can cause misleading conclusion if mistaken for the actual mtDNA loci (Zhang and Hewitt 1996b). Finally, as mitochondrial DNA is inherited through the maternal line, studies based on this type of marker ignore the paternal lineages, whose dispersal behavior might differ from their female counterparts (Meyer 1993).

Among nuclear markers, microsatellite DNA markers are the most widely used. They are also known as Simple Sequence Repeats (SSRs) or Short Tandem Repeats (STRs) and are co-dominant short di-, tri- or tetra-nucleotide tandem DNA repeatable sequences (Goldstein et al. 1995) inherited in a Mendelian way (Turnpenny and Ellard 2005). In the last decades they gained ground against mtDNA as they are i) hypervariable areas with high levels of polymorphism and mutational rates (Strassmann et al. 1996) as well as ii) highly accurate, requiring only small amounts of tissue (e.g. Goldstein and Schlotterer 1999; Avise et al. 2002; Garefallaki et al. 2010; Wilson and Veraguth 2010). In fish species they are widely used in the detection of inter- and intra- species relationships as well as in the study of parentage analysis (e.g. Kellogg et al. 1998; Jones and Avise 1997; Jones et al. 1999; Wilson 2009; Hubner et al. 2013) since allelic variation in most populations is extremely high (deWoody and Avise 2000).

3.1.2. Genetic structure of European populations of *Syngnathus* species

From a historical perspective, the first molecular- based study on syngnathids was published in the 1990s (Jones and Avise 1997). The phenomenon of male pregnancy brought syngnathids into the spotlight and molecular markers (microsatellites and mitochondrial markers) were used to assess their genetic mating system (e.g. Jones and Avise 1997, 2001; Mc Coy et al. 2001; Mobley and Jones 2009; Hubner et al. 2013) and phylogenetic relationships (e.g. Wilson et al. 2001; 2003; Lourie et al. 2005; Teske et al. 2005; Mobley et al 2010).

Phylogeographic studies on syngnathids revealed distinct patterns in their genetic architecture, including a) population structure over a restricted range (Lourie et al. 2005; Teske et al. 2005; Sanna et al. 2013), b) isolation by distance patterns (Lourie and Vincent 2004; Lourie et al. 2005; Wilson 2006b) and c) panmictic populations over large geographic distances (Lourie et al. 2005; Mobley et al. 2011). This variety in their modes of genetic structure constitutes syngnathid species as perfect model organisms to examine evolutionary phenomena (Mobley and Jones 2009; Mobley et al. 2011; Wilson and Orr 2011).

Most of the European pipefish - *Syngnathus abaster*, *Syngnathus nigrolineatus*, *Syngnathus rostellatus*, *Syngnathus taenionotus* and *Syngnathus typhle* - form a group of closely related taxa while *Syngnathus acus* is the most distinct (Wilson et al. 2001, Hablutzel 2009). From a molecular point of view, the most well- studied species of the European clade are *S. typhle* (Wilson and Veraguth 2010), *S. abaster* (Alaya et al. 2010; Sanna et al. 2013a) and *S. rostellatus* (Hablutzel and Wilson 2011). Only the first two species are widely found in the Mediterranean Sea (Dawson 1986).

The only so far existing study on the relative position of the European pipefish was conducted by Hablutzel (2009, master thesis), based on the mitochondrial control region and the nuclear Locus A1 (Figure 3.1). According to this study i) all species form distinct monophyletic clades with the exception of *S. typhle* which seems to be paraphyletic with *S. taenionotus* and ii) *S. abaster* is the first species to have diverged from the European pipefish clade. However, this study includes populations from a small fragment of the species range and therefore its results could not be easily generalized. Also the sequences produced are not available in GenBank (Benson et al. 2011) and therefore they could not be compared to the results of the present study.

In the most extended study of the European syngnathids genetic structure, Wilson and Veraguth (2010) provided the first phylogenetic scenario for *S. typhle* species. Based on the mitochondrial control region, the nuclear Locus A1 and nine microsatellite loci, they suggested that across most of the species' range four well-defined clades are formed, which reflect geographical distributions: i) North Sea and Atlantic coast, i) W. Mediterranean Sea, iii) Adriatic Sea and iv) Marmara and Black Seas (Figure 3.2). These clusters revealed the effect of Pleistocene glaciation events on northern and eastern *S.*

typhle populations, leaving the central ones intact. In particular, northern and eastern populations exhibited reduced genetic diversity and seem to have diverged 15,000-36,000 years ago. In contrast, central populations exhibited high levels of genetic diversity and their divergence times were estimated around 180,000–260,000 years ago, i.e. long before the last glacial cycle. Between these two extreme patterns, the Atlantic coast populations showed moderate levels of genetic variation and population structure, having diverged 60,000-80,000 years ago, i.e. in the last glacial cycle (Wilson and Veraguth 2010).

Two following studies shed light on the phylogenetic relationship of *S. abaster* species in the W. Mediterranean (Alaya et al. 2011; Sanna et al. 2013a). Both studies support the existence of well-defined clusters. In particular, Alaya et al. (2011) examined the genetic structure of *S. abaster* from two geographically distinct localities; Tunis north and Mauguio lagoon in Tunis and France respectively (Figure 3.3). They suggested that these populations formed two highly divergent and isolated groups probably due to repeated founder events and bottlenecks followed by re-expansion (Slatkin and Hudson 1991; Fu 1997; Alaya et al. 2011). For the analysis a combination of multiple loci were used: a 266 bp (base pairs) fragment of the 12S rDNA gene, a 519 bp fragment of the 16S rDNA gene, a 476 bp fragment of the Cytochrome b gene and a 458 bp fragment of the second segment of the control region.

The Sanna et al. (2013a) study was more comprehensive, as it included a wider sampling range (Figure 3.4). The analysis was based on a 458 bp fragment of the second hypervariable segment of the control region (HVS-II), a 266 bp fragment of the *12S r*RNA gene, a 519 bp fragment of the *16S r*RNA gene and a 476 bp fragment of the cytochrome b gene (*cyt b*). The results of this study indicated the occurrence of three distinct clusters of *S. abaster* populations: i) Spain, France, Corsica and Sardinia (Balearic, Sardinian and Ligurian Sea and Gulf of Lion) (cluster A), ii) the Italian mainland (cluster B) and iii) the Algeria- N. Tunisian (cluster C) (Figure 3.4). Divergence times indicated a more recent origin for the Tunisian group, while a similar one for the rest to two clusters (A and B). These calibration times suggested that the Pleistocene glacial period and more specifically the sea-level changes that occurred during that time played a pivotal role in the colonization pattern of *S. abaster*. The fact that *Syngnathus taenionotus* species grouped internally to *S. abaster s*uggests that *S. abaster* constitutes a paraphyletic taxon.

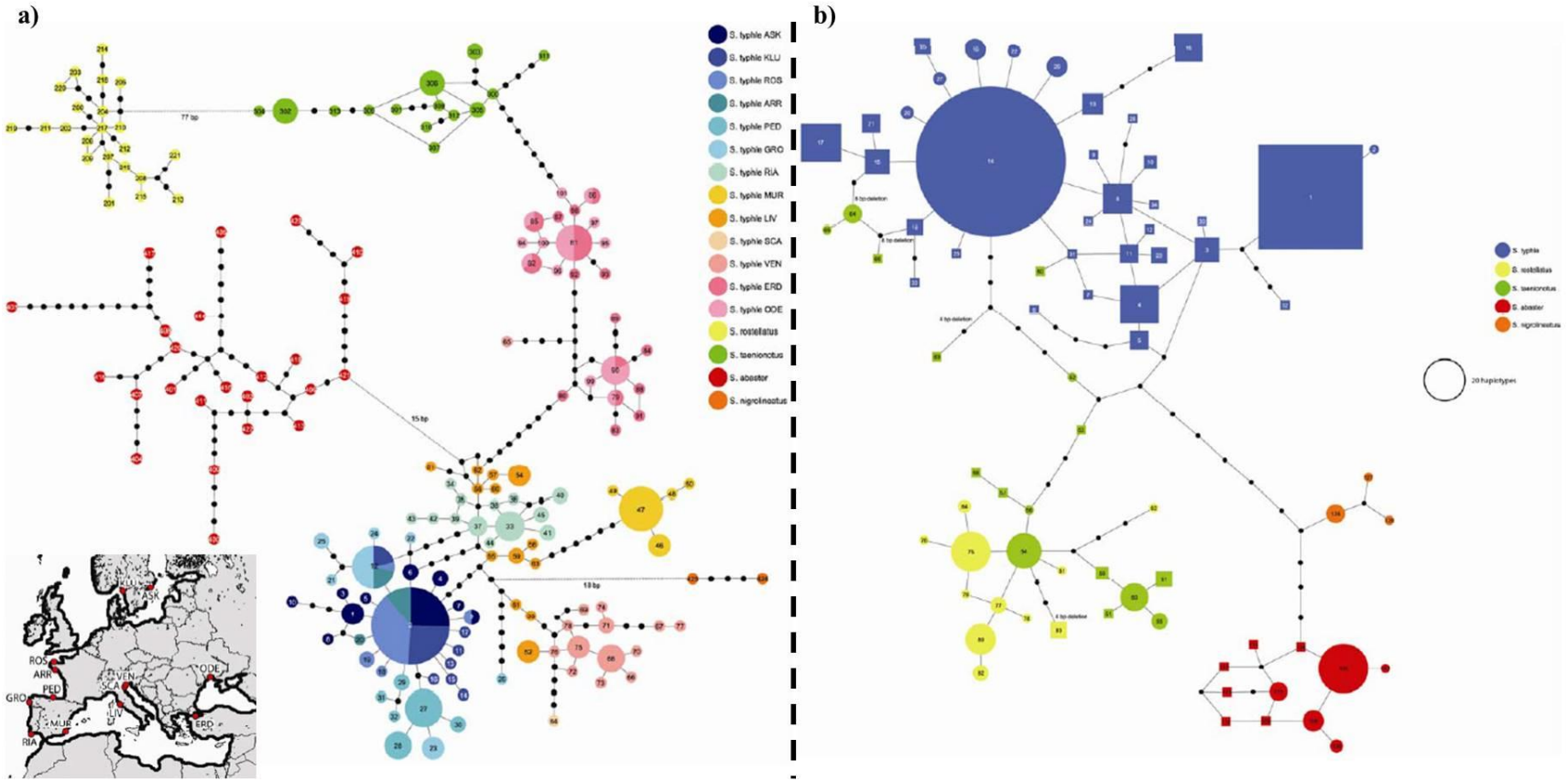

Figure 3.1. Unrooted haplotype network for a) mtDNA control region and b) nuclear Locus A1 of European Syngnathus species (modified after Hablutzel 2009).

Εικόνα 3.1. Δίκτυο απλοτύπων a) της περιοχής ελέγχου του μιτοχονδριακού DNA και b) του πυρηνικού τόπου A1 των ευρωπαϊκών ειδών του γένους *Syngnathus* (τροποποιημένο από Hablutzel 2009).

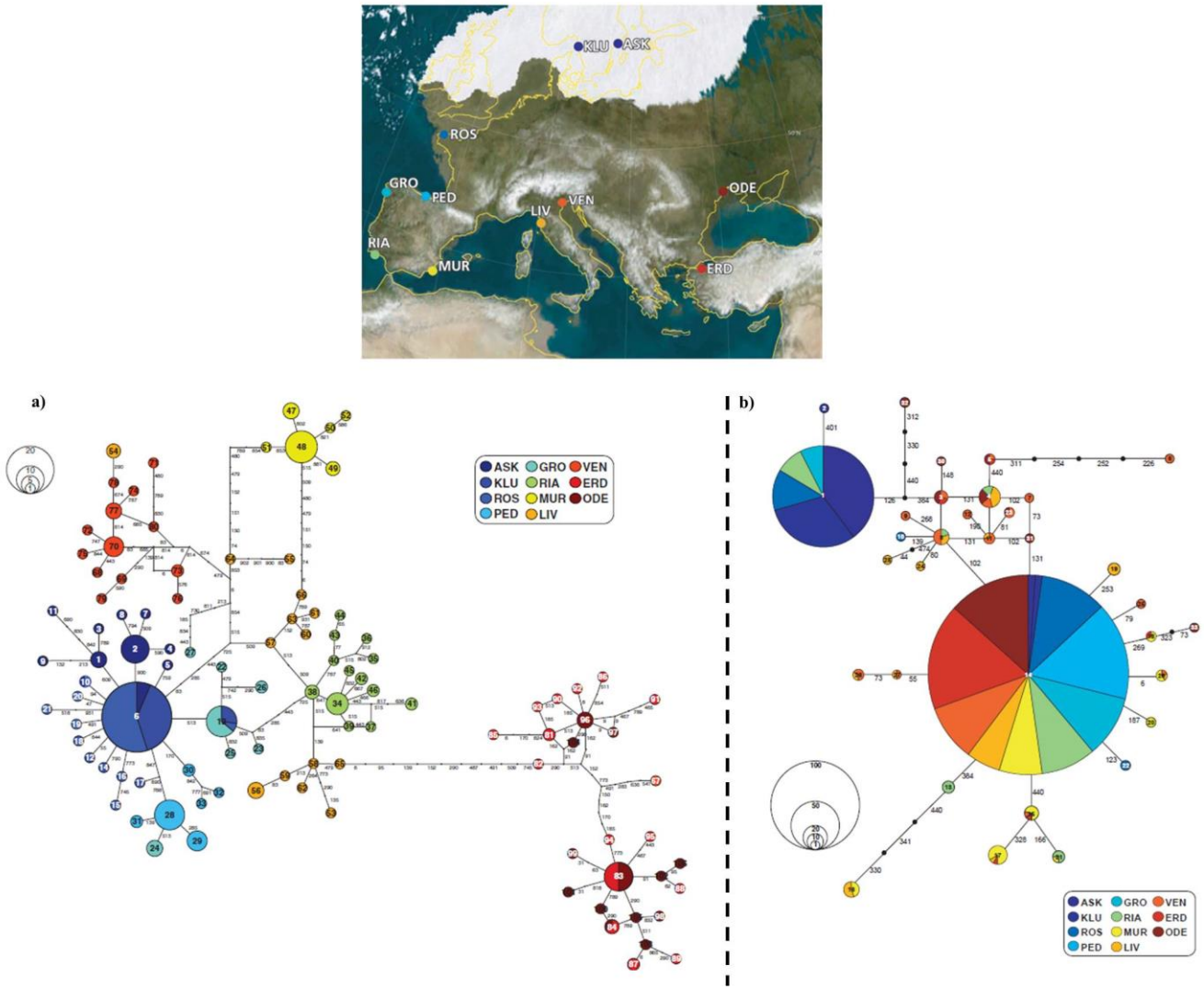

Figure 3.2. Map of *S. typhle* localities along the European coastline, and unrooted parsimony network of a) mtDNA control region and b) nuclear Locus A1 haplotypes. Unsampled intermediate haplotypes and SNPs defining haplotypes are indicated (modified after Wilson and Veraguth 2010).

Εικόνα 3.2. Χάρτης συλλογής δειγμάτων *S. typhle* κατά μήκος της ευρωπαϊκής ακτογραμμής και δίκτυο φειδωλότητας a) της περιοχής ελέγχου του μιτοχονδριακού DNA και b) του πυρηνικού τόπου A1. Οι ενδιάμεσοι απλότυποι και οι σημειακές μεταλλάξεις αποτυπώνονται στο σχήμα (τροποποιημένο από Wilson and Veraguth 2010).

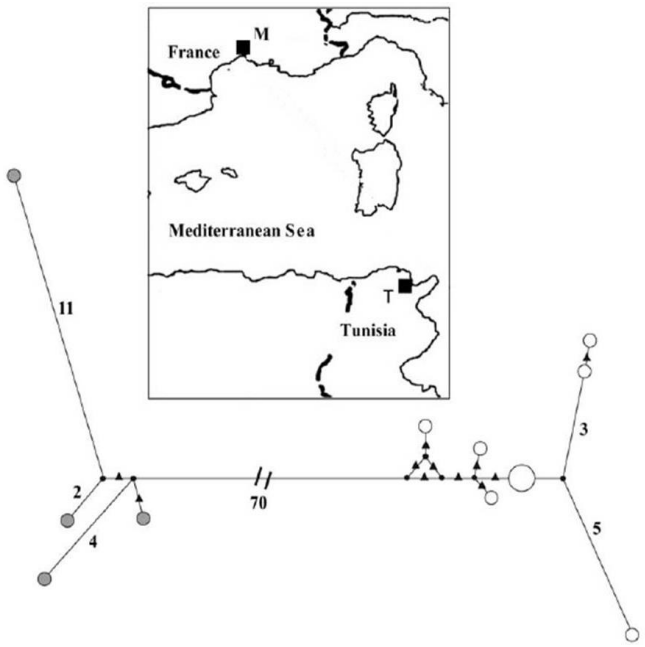

Figure 3.3. Median-Joining Network on the mitochondrial sequences dataset of populations of *S. abaster* from a Tunis (White spot) and French lagoon (Grey spots) sed. Black spots represent median vectors, arabic numbers number of mutations and black triangles one-point mutations (Modified after Alaya et al. 2011).

Εικόνα 3.3. Δίκτυο median-joining των σχέσεων των πληθυσμών του είδους *S. abaster* στη λιμνοθάλασσα της Τυνησίας (λευκός κύκλος) και της Γαλλίας (γκρι κύκλος). Οι μαύρες κουκίδες αντιστοιχούν στους ενδιάμεσους απλότυπους, οι αριθμοί στο πλήθος των μεταλλάξεων και τα μαύρα τρίγωνα σε σημειακές μεταλλάξεις (τροποποιημένο από Alaya και συν 2011)

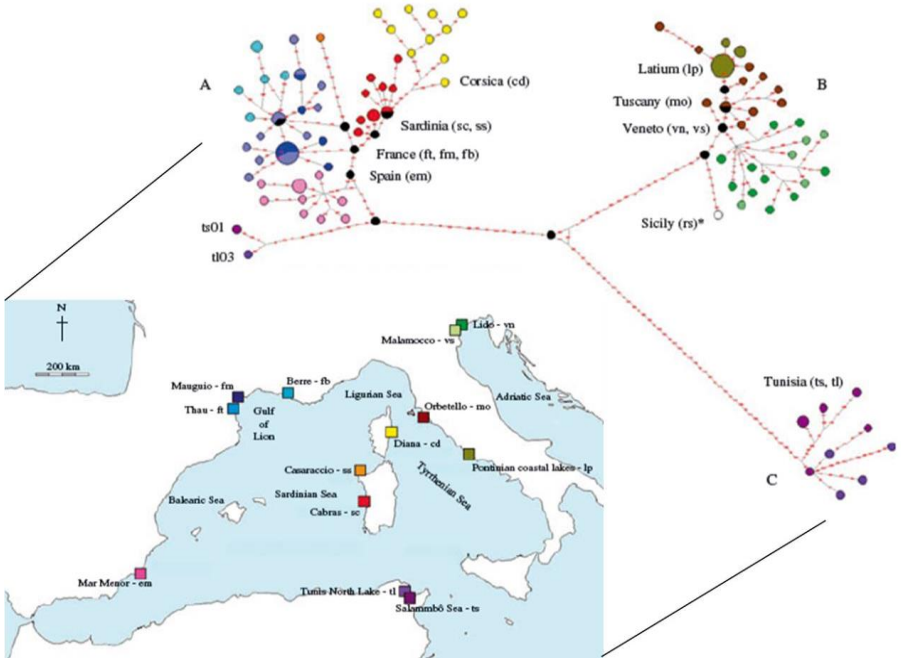

Figure 3.4. Median-joining network on the mitochondrial sequences dataset of populations of *S. abaster* from the W. Mediterranean. Mutating positions are shown in red (modified after Sanna et al. 2013a).

Εικόνα 3.4. Δίκτυο median-joining των σχέσεων των πληθυσμών της Δ. Μεσογείου του είδους *S. abaster*. Οι κόκκινες κουκκίδες αντιστοιχούν στον αριθμό των μεταλλάξεων (τροποποιημένο από Sanna και συν 2013a).

These studies add more information and start to unravel the puzzle of the two species' phylogeny. They reveal that, both species as near-shore animals were affected from the Pleistocene across their range. When the sea levels fell, they sought shelter in protected areas. When the sea levels rose again they managed to recolonize old and new habitats mostly through founders' events. As a consequence, at a European level both species exhibit isolation by distance dispersal pattern (Wilson and Veraguth 2010; Alaya et al. 2011; Sanna et al. 2013a). However, comparisons are not easy, since different molecular markers were employed and at different geographical areas.

As revealed by Figures 3.1- 3.4, the Ionian and Aegean Sea are unexplored territories in the phylogenetic map of both *S. abaster and S. typhle* species. This is a major gap in the two species phylogenetic overview as Ionian and Aegean Seas have a cornerstone position in the Mediterranean Sea. In particular, Ionian and Adriatic Seas - despite their geographical affinity- are recognized as distinct phylogeographic provinces for many Mediterranean taxa (e.g. Gysels et al. 2004; Bianchi 2007; Patarnello et al. 2007; Coll et al. 2010). At the same time, the Aegean Sea is connected with the Marmara and the Black Seas through the Straits of Vosphoros and Dardaneiles (Aksu et al. 1995). Finally, Ionian/ S. Aegean Sea and N. Aegean Seas constitute two different biogeographical regions (Bianchi et al. 2007, 2012), whose formation is attributed to Pleistocene events (Chronis et al. 1991; Aksu et al. 1995; Perissoratis et al. 2000; Lykousis 2009).

Phylogenetic analyses of marine organisms whose population structure was also affected by the postglacial events reveal contrasting patterns between the Ionian and Aegean Seas. The most commonly reported break within the Mediterranean is the Sicilo-Tunisian which separates Western and Eastern populations leading to a unified Aegean-Ionian clade (e.g. Stefanni and Thorley 2003; Karaiskou et al. 2004; Zardoya et al. 2004). However, in some species within the Mediterranean Sea an eastward shift to the geographic position of the genetic break has been reported indicating southern Peloponnese (S. Aegean Sea) as a more possible location for the break instead of the Sicilo-Tunisian Straits. In this case Ionian and Aegean Sea populations form distinct clades (e.g. Magoulas et al. 1996; Nikula and Vainola 2003; Costagliola et al. 2004, Domingues et al. 2005). These scenarios indicate that a species genetic structure within Ionian and Aegean cannot be taken a priori for granted but instead should be species- specifically examined.

Besides past demographic events, contemporary drives (environmental parameters or/and species-specific life history/ecological traits) also determine species phylogenetic relationships (Avise, 2000, Hewitt 2000; Zardoya et al. 2004; Pujolar et al. 2011, Palumbi 1994, Vanhove et al. 2012). *S. abaster* and *S. typhle* despite congeneric and co-occurring species exhibit different life history traits (e.g., tolerance to salinity, vertical position, early life history stages and lifespan) (e.g. Berglund et al. 1986, Vincent et al. 1995, Franzoi et al. 1993, Silva et al. 2006a, Miersch 2012). These differences could also affect their potential dispersal rate and shape their genetic structure.

Under this perspective, the first goal of the present study was to examine the genetic structure of *S. abaster* and *S. typhle* species in the Greek coastline and test whether the Ionian and Aegean Seas populations form distinct clades or not. It was hypothesized that the biogeographical differentiation of the two Seas (Bianchi et al. 2007) combined with the species restricted migration ability (due to the absence of pelvic fin and the presence of rigid bony plates all over the body) would prevent population exchange between remote areas (Storz 1999; Ross 2001). Therefore, isolated populations in the Ionian and Aegean Seas were expected to be found. Also, it was hypothesized that the recorded difference in the dispersal ability of the two species would affect their degree of isolation. Particularly, as *S. abaster* juveniles assume benthic position right after they are born and retain that position as adult specimens they are less susceptible to active migration and passive transportation (Silva et al. 2006). On the contrary, the pelagic juveniles of *S. typhle* are more susceptible to passive transportation (Berglund et al. 1986). Therefore, within the Ionian and Aegean Sea populations a shallower degree of genetic structuring in *S. typhle* species and a higher in *S. abaster* was expected to be found.

Moreover, phylogenetic analysis of *S. typhle* species indicated that within the so far studied Mediterranean populations three distinct clades were formed which are in accordance with existing biogeographical zones; W. Mediterranean, Adriatic and Marmara-Black Sea (Wilson and Veraguth 2010). As already stated, in that study there is no available information on the genetic structure of the E. Mediterranean Sea population or its relationship with the rest of the European populations. Numerous studies on a broad range of Mediterranean species indicate that their populations cluster into Western and Eastern Mediterranean clades (e.g. Zardoya et al. 2004 Magoulas et al. 1996; Nikula and Vainola 2003; Costagliola et al. 2004, Domingues et al. 2005; Sanna et al. 2013b). At the same time most species Ionian Sea populations are usually genetically distinct from the Adriatic Sea's (e.g. Tinti et al. 2002; Steffani and Thorley 2003, Garoia et al. 2004; Triantafyllidis et al. 2005; Rolland et al. 2007). Finally, the relationship of Aegean Sea and Black Sea populations is species-specific as both unified and distinct clades of the populations of the two Seas have been reported (e.g. Ladoukakis et al. 2002, Nikula and Vainola 2003; Suzuki et al. 2004; Magoulas et al. 2006; Yebra et al 2011).

Taking the above facts into consideration, the second goal of the present study was to place the Ionian and Aegean Sea populations of *S. typhle* into the European-PontoCaspian species tree and test whether they group with the W. Mediterranean, Adriatic or/and Marmara-Back Sea populations or form a clade of their own; the E. Mediterranean. The Greek population was expected to be distinct from the Adriatic Sea clade. According to the results of the genetic population analysis a certain degree of differentiation was expected to be found with the W. Mediterranean clade. In particular, if Ionian and Aegean Sea populations formed distinct clades, Ionian populations were expected to group within the W. Mediterranean Sea clade. On the contrary, a panmictic Greek population of *S. typhle* would group Ionian and Aegean Sea population into the E. Mediterranean clade, separating it thus from the W. Mediterranean. Also, the relationship of the Aegean Sea

population to the Marmara- Black Sea complex could not be a priori estimated as it is species-specific (e.g. Ladoukakis et al. 2002, Nikula and Vainola 2003; Suzuki et al. 2004; Magoulas et al. 2006; Yebra et al 2011).

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3.2. MATERIALS AND METHODS

3.2.1. Sampling stations

Specimens of *S. abaster* (n = 142) and *S. typhle* (n = 38) were collected from June to August 2010, along the mainland sublittoral coastline of Greece (Figure 3.5, Table 3.1. in Appendix), using a hand net (mesh size of 2–4 mm). Samples were stored at 80% alcohol solution. The 19 localities depicted the range of the species distribution along the major biogeographical basins in the coastline of the mainland Greece. In the laboratory, each specimen was identified to the species level based on a key for European syngnathids (Dawson 1986).

Figure 3.5. Map of Greece showing the sampling stations for the two studied species in the present study (● *S. typhle* Ionian Sea, ● *S. typhle* Aegean Sea, ● *S. abaster* Ionian Sea, ● *S. abaster* Aegean Sea). For number of samples see Table 3.1 in Appendix.

Εικόνα 3.5. Περιοχές δειγματοληψίας στην Ελλάδα για τα δύο μελετώμενα είδη κατά τη παρούσα μελέτη (● *S. typhle* Ιονίου Πελάγους, ● *S. typhle* Αιγαίου Πελάγους, ● *S. abaster* Ιονίου Πελάγους, ● *S. abaster* Αιγαίου Πελάγους). Ο αριθμός των συλλεχθέντων ατόμων παρουσιάζεται στον Πίνακα 3.1 του παραρτήματος

3.2.2. DNA extraction

Genomic DNA from all specimens was extracted from tail muscle tissue after the modified phenol-chloroform extraction protocol (Sambrook and Russel 2001). The procedure is as follows:

1. Put 100-200 mg of tissue in a 1-2 ml Eppendorf tube.
2. Add 500 µl CTAB (Cetyl Ttimethylammonium bromide) Buffer (2 x).
3. Add 10 µl Proteinase K (10 mg/ml).
4. Vortex to mix well.
5. Incubate in water bath at 55 ^{o}C for at least 2-4 hours.
6. Add 250 µl phenol and 250 µl chloroform-isoamyl alcohol (24:1) solution.
7. Vortex thoroughly.
8. Rotor for about 10 minutes at room temperature.
9. Spin at 13000 rpm for 10 minutes.
10. Have new sterile tubes labeled and ready.
11. Transfer the aqueous solution into the new eppendorfs.
12. Repeat steps 9-14 one more time.
13. Add 250 µl chloroform-isoamyl alcohol.
14. Vortex thoroughly.
15. Rotor for about 10 minutes at room temperature.
16. Spin at 13000 rpm for 10 minutes.
17. Have new sterile tubes labeled and ready.
18. Transfer the aqueous solution into the new eppendorfs.
19. Add 1000 µl ice-cold ethanol.
20. Keep in the freezer overnight.
21. Spin at 13000 rpm for 15 minutes.
22. Pour out the ethanol. The DNA is at the bottom of the tube.
23. Add 1000 µl of 70% ethanol.
24. Rotor for at least one hour.
25. Pour out ethanol.
26. Keep at 37 ^{o}C for about 20 minutes to evaporate the alcohol.
27. Add about 100 µl TE buffer.
28. Store in the freezer overnight.
29. Store in the freezer until use.

The amount of DNA extracted was quantified by loading 5 µl of each extraction on a 1 % agarose gel stained with ethidium bromide.

3.2.3. Mitochondrial and nuclear DNA amplification and sequencing

3.2.3.1. Mitochondrial DNA amplification

A 932 bp sequence of the mitochondrial CR (including tRNA-Phe and a short fragment of 12SrDNA) was amplified for two to ten individuals from each sampling station. LPRO (forward, 5'-AACTCTCACCCCTAGCTCCCAAG-3', Meyer *et al.*, 1994) and 12Sr.5 (reverse, 3'-GGCGGATACTTGCATGT-5', Hrbek and Farias 2008) were used as primers.

3.2.3.2. Nuclear DNA amplification

PCR primers SlepA1F (forward, 5'-ATCTGAGCCAGCGGGCCGAGCAG-3') and SlepA1R (reverse, 3'-TGGAGCGCGGCTTGCAGTCGTG-5') (Wilson and Veraguth 2010) were used to amplify the nuclear region LocusA1 (490 bp), which is a microsatellite-flanking sequence (Wilson and Veraguth 2010). The neighboring microsatellite locus was excluded from the study (Wilson and Veraguth 2010). One to five individuals were amplified in each station.

3.2.3.3. Polymerase Chain Reactions and Sequencing

PCRs were performed in 25 μl volume reactions containing 5U KAPA Taq ReadyMix (KAPABIOSYSTEMS), 25 mM $MgCl_2$, 100 pmol primers and 100 ng DNA. Negative (2.5 μl ddH_2O instead of DNA) and positive (100 ng DNA of an individual whose CR or A1 locus had already been successfully amplified) controls were included in all reactions. PCR cycles were performed on an MJ Research PTC- 200 gradient cycler under the conditions shown in Table 3.1. To check for amplification, PCR products were visualized in 1.5% agarose gels. Successful reactions were purified with Nucleospin® Extract II kit (Macherey-Nagel) and eluted in 50 μL volume. The mtDNA and nDNA purified products were sent for sequencing to VBC Biotech and Beckman Coulter service providers respectively.

Table 3.1. PCR conditions for the mitochondrial control region and the nuclear locus A1 used in the present study.
Πίνακας 3.1. Συνθήκες που ακολουθήθηκαν στις αντιδράσεις PCR για την περιοχή ελέγχου του μιτοχονδριακού DNA και του πυρηνικού τόπου A1κατά τη διάρκεια της παρούσας μελέτης.

	mtDNA, Control Region	**nDNA, Locus A1**
Step1. Initial denaturation	94 °C for 180 sec	95 °C for 180 sec
Step2. Denaturation	94 °C for 30 sec	94 °C for 30 sec
Step3. Annealing temperature	56 °C for 30 sec	68 °C for 30 sec
Step4. Extension	72 °C for 60 sec	72 °C for 30 sec
Step5.	repetition of steps 2-4 for 30 cycles	repetition of steps 2-4 for 30 cysles
Step6. Final extension	72 °C for 300 sec	72 C for 120 sec

3.2.4. Sequences retrieved from GenBank

Sequences available for European populations of *Syngnathus typhle* were retrieved from GenBank (Benson et al. 2011) and incorporated with sequences from this study in order to have a deeper insight into the phylogeography of the species (Accession number for mtDNA: HM773035- HM773140; Accession number for nDNA: HM773141–HM773173) (Wilson and Veraguth 2010).

3.2.5. Data analyses

3.2.5.1. Mitochondrial DNA

Raw sequences were manually edited with Geneious 5.4 software (Drummond et al. 2010) using the default parameters. As the beginning and the end of the sequence could often not be reliably scored the first 51 bp and the final 65 bp were not included, yielding a total sequence length of 816 bp for all samples. Sequence alignment was performed using the ClustalW 2.1 algorithm (Thomson et al. 1994). Parameters taken into consideration for multiple alignment were gap opening = 15.00 and gap extension = 6.66.

The ARLEQUIN 3.5 software (Excoffier and Lischer 2010) was used to estimate intrapopulation diversity for each species by estimating the number of polymorphic sites (S), the number of haplotypes (Hp), the occurrence of shared and unique haplotypes, nucleotide diversity (π; Nei 1987) and haplotype (gene) diversity (H; Nei & Tajima 1981):

- Nucleotide diversity is the probability that two randomly chosen homologous nucleotides are different. One commonly used measure is the "*average number of nucleotide differences per site between any two DNA sequences chosen randomly from the sample population;* π" (Nei 1987). It is similar to expected heterozygosity and is given by the formula:

$$\hat{\pi} = \frac{n}{n-1} \sum_{i=1}^{k} \sum_{j=1}^{k} p_i p_j \hat{d}_{ij}$$

  Where x_i and x_j are the respective frequencies of the i-th and j-th sequences, π_{ij} is the number of nucleotide differences per nucleotide site between the ith and jth sequences, and n is the number of sequences in the sample.

- Haplotype (gene) diversity (H) is defined as the probability that two randomly chosen haplotypes are different in the sample and is given by the formula:

$$\hat{H} = \frac{n}{n-1}(1 - \sum_{i=1}^{k} p_i^2)$$

where, n is the number of gene copies in the sample, k is the number of haplotypes, and p_i is the sample frequency of the i-th haplotype.

The overall number of polymorphic sites (S), number of haplotypes (Hp) nucleotide diversity (π; Nei, 1987) and haplotype (gene) diversity (*H*; Nei & Tajima 1981) for each species were estimated by the software DnaSP 5.0 (Librado and Rozas 2009).

In order to investigate the geographical structuring of individuals, Analysis of Molecular Variance (AMOVA) was implemented (Excoffier et al. 1992) using ARLEQUIN 3.5 (Excoffier and Lischer 2010). AMOVA partitions the total genetic variation into variance component and leads to quantities of genetic distance among and within populations analogous to classical *F*-statistics'; Fixation indices (Weir and Cockerham 1984). AMOVA estimates the proportion of variation among groups (F_{CT}), the proportion of variation among populations within groups (F_{SC}), and the proportion of variation within populations (F_{ST}). The significance of these F-statistic analogs is evaluated by random permutations of sequences among populations. This procedure is more suitable when dealing with uneven sample sizes, as it does not assume normality or equality of variances among samples. Therefore, pairwise Fst was calculated for all populations together and separately for each species. Various groupings of populations were tested (1. Sampling stations, 2. Regions, 3. Ionian- Aegean Seas). Groupings that maximize values of F_{CT} and are significantly different from random distributions of individuals are assumed to be the most probable geographic subdivisions. Departures from the null hypothesis of panmixia were evaluated via a permutation test of 10100 iterations.

In order to examine the genetic relationships between the identified haplotypes a second data set was assembled by retaining only one sequence per haplotype. An alignment was constructed using the parameters described above. The software MODELTEST 3.7 (Posada and Crandall 1998) was used to specify the best model of sequence evolution that fits the data for each species and to calculate the proportion of invariable sites and the value of the gamma distribution shape parameter, on the basis of BIC (Bayesian information criterion) values. After model selection, genetic distances between and within Greek populations of *S. abaster* and *S. typhle* species were computed in MEGA 6.0 software (Tamura et al. 2013). A bootstrap test (Felsenstein 1985) of 1000 replicates was carried out to check the strength of each branch of the tree. The resulting trees were edited with FigTree software (http://tree.bio.ed.ac.uk/software/figtree/).

In order to further elucidate the relationships between i) syngnathids from the littoral coastline of Greece, ii) European population of *S. typhle* species and iii) Greek populations of *S. abaster* and European populations of *S. typhle*, unrooted haplotype

networks were reconstructed using the software Network 4.6.1.2 (Fluxus Technology, www.fluxus.engineerring.com). A median joining algorithm was used. Equal weights for all mutations were assumed and the genetic distance parameter "e" was set to zero in order to restrict the choice of feasible links in the final network. Haplotypes were estimated and converted to the appropriate format (roehl file format) using the DnaSP 5.0 software (Librado and Rozas 2009).

3.2.5.2. Nuclear DNA

Raw data were analyzed and aligned by eye using Geneious 5.4 (Drummond et al. 2010). As the beginning and the end of the sequence could often not be reliably scored the first 29 bp and the final 25 bp were not included, yielding a total sequence length of 438 bp for all samples.

Heterozygous sites in nDNA genotypes were coded using IUPAC nomenclature for degenerate positions and allelic sequences were inferred using the default settings of PHASE V2.1.1 (Stephens and Donnelly 2003). The program implements a Bayesian statistical method for reconstructing haplotypes from population genotype data, under the assumption that sequence data are provided without error (Stephens et al. 2001). This method is highly accurate in inferring alleles that are well-represented in a diploid data set but may not be able to reconstruct allelic phase for unique genotypes (Harrigan et al. 2008). This however was not the case as all phase probabilities in the present study were greater than 0.63 indicating the correct phase (Harrigan et al. 2008).

All the other analyses -diversity of nuclear DNA alleles, geographical structuring of population (AMOVA and Fixation indices), model of sequence evolution that best fits the data, genetic distances and unrooted haplotype networks- between and within Greek individuals of *S. abaster* and *S. typhle* species, as well as the unrooted haplotype networks of European and Greek specimens of *S. typhle* were calculated as described for the mitochondrial DNA data.

3.3. RESULTS

3.3.1. *Syngnathus abaster* species

3.3.1.1. mtDNA Control Region

The mitochondrial DNA control region of Greek population of *S. abaster* species revealed 67 variable sites (Figure 3.6, Table 3.2.a in Appendix). A total of 78 haplotypes were detected in a 816 bp fragment sequenced for 115 individuals from 19 sampling stations (Figure 3.7, Table 3.3.a in Appendix). The level of nucleotide diversity for the entire sample was $\pi = 0.016 \pm 0.002$ (ranging from 0 in individuals from Kotichi station to 0.015 in Kalogria station), while the haplotype diversity was $h= 0.990 \pm 0.003$ (ranging from 0 in individuals in Kotichi station to 1.000 in Neochori, Tourlida, Karavomilos, Korinnos and Vassova stations) (Table 3.2).

TTGAGTTATTTAAA**C**CACAGAACTAAG**A**CATT**C**CACTAAG**A**TAT**C**CATAA	50
ACCATTAATGCAAACTA**C**TAAAT**C**TTAGTTTAGT**G**TTA**T**TGGTTCCTAA**G**	100
A**C**CTAACACAA**C**ACTCATA**A**GTTAAGTTATACCA**C**GACTCCAAA**A**TCGAT	150
TAAATTAAGTAT**C**TTAATGTAGTAAGAGCCTACCTACCAGTCCATTTCTT	200
AATGCCAACGGTTATTGATGGTCAGGCGCCCTTATTGTG**A**GGGTAGCTAC	250
CTAAAAGGTGAATTATTCCTGGCATTTGG**C**TCCTACTTCAGGTCCATTAA	300
TTTATTAACCCTCGCACTTTCATCGACGCTAGCATAAGTTAATGGTGGAA	350
ATCATACGACTCGTTACCCCCCAAGCCGGGCGTTCACTCCAC**G**GGGGCAG	400
CTGGTTCCTTTTTTC**G**TTTTCCTTTCA**CT**TAGCATTTCA**G**AGTGCACACG	450
GTATTAG**A**TGA**T**AAGGTTGAACATTTCCTTGAATGAGTATATTCGTT**T**AA	500
TGTTGGAAAGACATTACATAAGAATTGCATATATCTATTACTAAAGCATA	550
ATACCTAAATTTTAGTCCTAATATTTTTAAGAT**CG**CCCCTTCTTGGTTTA	600
ATTCCGACAAACCCC**C**TACCCCTTACAA**C**C**C**T**G**ACATGT**CCAT**CACTCCT	650
GCAAACCCCTAAGAAACAGGAA**T**GT**TCCGA**GTAAAACTT**ATT**T**G**T**C**TCACT	700
CGTCAATCAACAAATGTCATATGT**A**TATAGT**A**T**TG**T**T**A**G**ATTTTCAAAA**T**	750
A**C**ACC**T**GTAT**GCCATA**TTT**A**AC**T**CATT**A**GACTCAAATTA**C**ACTA**A**GACCT	800
ACT**C**GCTGTTGTAGCT	816

Figure 3.6. DNA consensus sequence of a 816 bp amplified segment of mitochondrial Control Region. Variable positions revealed after the analysis of 115 individuals of Greek *S. abaster* species during the present study are bold and underlined.

Εικόνα 3.6. Ακολουθία αναφοράς του ενισχυμένου τμήματος της περιοχής ελέγχου του μιτοχονδριακού DNA (816 ζ.β.). Οι πολυμορφικές θέσεις που προέκυψαν από την ανάλυση 115 ατόμων του είδους *S. abaster* στον ελλαδικό χώρο κατά τη διάρκεια της παρούσας μελέτης, παρουσιάζονται έντονες και υπογραμμισμένες.

The two most abundant haplotypes were haplotype 1 and haplotype 10 (Table 3.3.a. in Appendix). The number of haplotypes in individuals from each station ranged from one to 11 (individuals from Kotichi and Korinnos stations respectively). The most polymorphic individuals were the ones from Kalogria station (Ionian Sea, Peloponnesus) with 37 polymorphic sites (Table 3.2). The stations with the higher number of haplotypes were Korinnos (11) and Kalogria (10). However, Korinnos' haplotypes were all station-specific, whereas Kalogria's were shared with individuals from three additional stations (Kotichi, Vassova and Drana Tables 3.2, 3.3.a. in Appendix).

The high percentage of the total number of haplotypes divided by the number of individuals observed in most stations (V) is an indicator of the species polymorphism; in most cases each individual corresponds to one haplotype. However, it is also evident that more individuals need to be examined in order to have a clearer view of haplotype frequencies. As revealed by the number of unique haplotypes (UH) and the percentage of the station-specific haplotypes divided by the number of individuals (W) there was a striking lack of haplotype sharing among stations and between the Ionian and Aegean Seas (Tables 3.2, 3.3.a. in Appendix). The exception to this pattern was the specimens from the Kalogria and Kechries stations.

Table 3.2. Genetic variability observed in the mitochondrial DNA control region of Greek individuals of *S. abaster* species, as revealed in the present study (*n*, sample size; *Hp*, number of haplotypes; *UHp*, station- specific haplotypes; *V*, the percentage of the total number of haplotypes divided by the number of individuals; *W*, the percentage of the station-specific haplotypes divided by the number of individuals; *S*, number of polymorphic sites; *h*, haplotype diversity; *π*, nucleotide diversity) (values in round brackets correspond to standard deviation values).
Πίνακας 3.2. Γενετική ποικιλότητα της περιοχής ελέγχου του μιτοχονδριακού DNA των ατόμων του είδους *S. abaster* στον ελλαδικό χώρο, με βάση την παρούσα μελέτη (*n*, ο αριθμός των ατόμων; *Hp*, οι απλότυποι των ατόμων από κάθε σταθμό δειγματοληψίας; *UHp*, οι μοναδικοί απλότυποι κάθε σταθμού; *V*, το ποσοστό συνολικού αριθμού απλοτύπων/αριθμό ατόμων; *W*, το ποσοστό μοναδικών απλοτύπων/αριθμό ατόμων; *S* αριθμός πολυμορφικών θέσεων; *h*, η απλοτυπική ποικιλότητα; *π*, η νουκλεοτιδική ποικιλότητα) (οι τιμές στις παρενθέσεις αντιστοιχούν στην τυπική απόκλιση).

Sampling Station	n	Hp	UHp	V (%)	W (%)	S	h (±)	π (±)
1.Drepano	8	4	2	50.00	25.00	8	0.643 (±0.184)	0.003 (±0.002)
2.Neochori	4	4	4	100.00	100.00	6	1.000 (±0.177)	0.004 (±0.003)
3.Mitikas	10	5	5	50.00	50.00	6	0.667 (±0.163)	0.001 (±0.001)
4.Tourlida	6	6	5	100.00	83.33	12	1.000 (±0.096)	0.006 (±0.004)
5.Kalogria	11	10	5	90.91	45.45	37	0.982 (±0.046)	0.015 (±0.008)
6.Katakolo	3	2	1	66.67	33.33	1	0.667 (±0.314)	0.001 (±0.001)
7.Kotichi	2	1	0	50.00	0	0	0	0
8.Kaiafa	5	2	2	40.00	40.00	1	0.600 (±0.175)	0.007 (±0.008)
9.Moustos	5	3	3	60.00	60.00	2	0.700 (±0.218)	0.001 (±0.001)
10.Kechries	4	3	3	75.00	75.00	16	0.833 (±0.222)	0.010 (±0.007)
11.Livanata	3	2	1	66.67	33.33	6	0.667 (±0.314)	0.005 (±0.004)
12.Karavomilos	7	7	7	100.00	100.00	22	1.000 (±0.076)	0.013 (±0.008)
13.Lechonia	5	3	3	60.00	60.00	14	0.700 (±0.218)	0.010 (±0.006)
14.Korinnos	11	11	11	100.00	100.00	16	1.000 (±0.039)	0.006 (±0.004)
15.Pilaia	6	5	5	83.33	83.33	13	0.933 (±0.122)	0.006 (±0.004)
16.Vourvourou	4	3	2	75.00	50.00	8	0.833 (±0.222)	0.006 (±0.004)
17.Porto Koufo	8	5	5	62.50	62.50	15	0.786 (±0.151)	0.005 (±0.003)
18.Vassova	6	6	3	100.00	50.00	22	1.0000 (±0.096)	0.012 (±0.007)
19.Drana	6	5	2	71.43	28.57	11	0.952 (±0.096)	0.005 (±0.003)

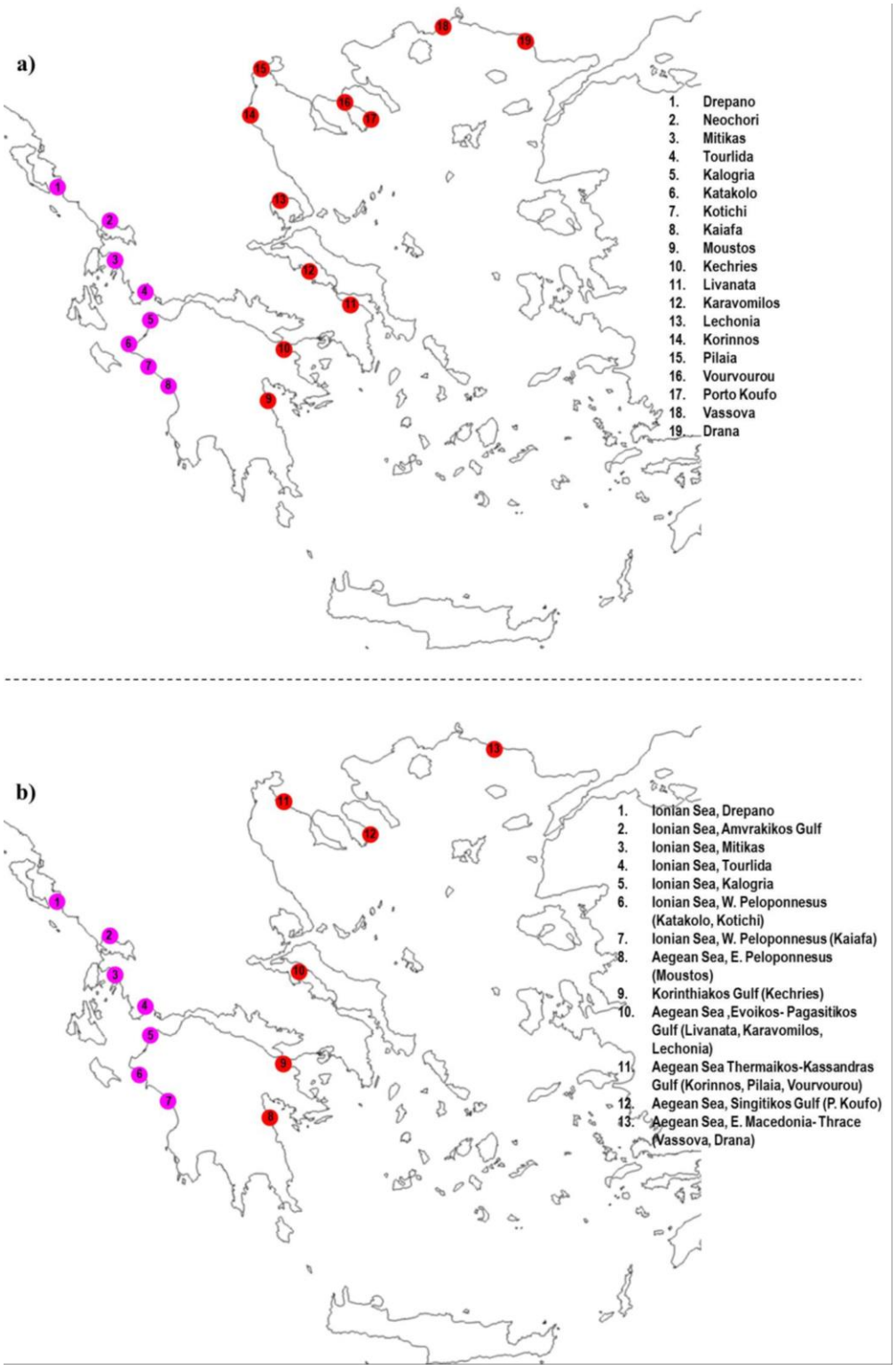


Figure 3.7. Depiction of sampling stations of *S. abaster* species along the sublittoral zone of Greece; a) 19 sampling stations, (b) 13 regions based on non-significance of pairwise values of fixation indices (see Table 3.5).

Εικόνα 3.7. Απεικόνιση των σταθμών του είδους *S. abaster* κατά μήκος της ελληνικής υποπαραλιακής ζώνης σε (a) 19 σταθμούς δειγματοληψίας, και (b) 13 περιοχές, ομαδοποιημένες με βάση τις μη στατιστικά σημαντικές τιμές F_{ST} ανά ζεύγος πληθυσμών (βλ. Πίνακα 3.5).

The results of the AMOVA analysis of the individuals from the 19 initial samples resulted in the rejection of the homogeneity hypothesis. The level of differentiation was very high (*Fst* = 0.604; P < 0.001) (Table 3.3.a), i.e. 60.43% of variation was attributed to among-station differences. Subsequently, the 19 sampling stations were grouped in 13 regions according to the results of the pairwise comparison (Table 3.4 and 3.5, Figure 3.7.b). AMOVA analysis based on this grouping led to the highest F_{CT} values (F_{CT} = 0.534; P < 0.000) (Table 3.3.b). Therefore this grouping was highly successful since most of the among-station differences ware due to the among-group differences (53.39%) instead of the among-population within groups (7.9%) (Table 3.3.b). When trying to divide specimens in a simple Aegean / Ionian group scheme, the F_{CT} value was lower (F_{CT} = 0.359; P < 0.001), indicating that even though there is highly significant distinction between the individuals of the two Seas the grouping of samples into 13 regions is a better depicter of the distribution of genetic variability among *S. abaster* populations (Table 3.3.c).

Table 3.3. Results of the hierarchical analysis of molecular variance (AMOVA) for *S. abaster* species for the (a) 19 sampling stations; (b) 13 grouped regions; (c) Ionian-Aegean Sea populations, based on mtDNA Control Region.
Πίνακας 3.3. Αποτελέσματα της ιεραρχικής ανάλυσης μοριακής διακύμανσης (AMOVA) του είδους *S. abaster* για: (a) 19 σταθμούς δειγματοληψίας, (b) 13 ομαδοποιημένες περιοχές, (c) πληθυσμούς Ιονίου- Αιγαίου, με βάση την ανάλυση της περιοχής ελέγχου του μιτοχονδριακού DNA.

Grouping	Source of Variation	Percentage of Variation	Fixation Indices	P values
(a)	Among stations	60.43	F_{ST}= 0.604	P < 0.001
	Within stations	39.57		
(b)	Among groups	53.39	F_{CT} = 0.534	P < 0.001
	Among stations within groups	7.90	F_{SC} = 0.170	P < 0.05
	Within stations	38.71	F_{ST} = 0.613	P < 0.001
(c)	Among stations	35.94	F_{CT} = 0.359	P < 0.001
	Among stations within groups	31.30	F_{SC} = 0.359	P < 0.001
	Within stations	32.77	F_{ST} = 0.672	P < 0.001

Table 3.4. Values of exact tests for differentiation among the individuals of *S. abaster* from the 19 sampling stations based on mtDNA Control Region.
Πίνακας 3.4. Τιμές του ελέγχου διαφοροποίησης ανά ζεύγος του είδους *S. abaster* με βάση την ανάλυση της περιοχής ελέγχου του μιτοχονδριακού DNA.

	Drepano	Neochori	Mitikas	Tourlida	Kalogria	Katakolo	Kotiki	Kaiafa	Moustos	Kechries	Livanata	Karavomilos	Lechonia	Korinnos	Pilaia	Vourvourou	P. Koufo	Vassova	Drana
Drepano	0.00000																		
Neochori	0.71171*	0.00000																	
Mitikas	0.47387**	0.75189*	0.00000																
Tourlida	0.34660*	0.53671*	0.30602*	0.00000															
Kalogria	0.51782**	0.50273**	0.52782**	0.42391*	0.00000														
Katakolo	0.50757*	0.74634*	0.49134*	0.28144	0.43477*	0.00000													
Kotiki	0.21778	0.76361	0.71698*	0.37017*	0.40243*	0.90551	0.00000												
Kaiafa	0.76520**	0.81195*	0.78860**	0.18164*	0.48678**	0.89734*	0.93576*	0.00000											
Moustos	0.91067**	0.91677*	0.93969**	0.83509*	0.56766**	0.96040*	0.96877*	0.96067*	0.00000										
Kechries	0.67002*	0.62993*	0.70655*	0.53885*	0.29292*	0.59609	0.52941	0.71162*	0.72394*	0.00000									
Livanata	0.86601*	0.84530*	0.90609*	0.76914*	0.07063	0.88068	0.87629	0.91080*	0.90402*	0.62590*	0.00000								
Karavomilos	0.64485**	0.60173*	0.66874**	0.54539**	0.04126	0.54507*	0.52323*	0.62513*	0.58980*	0.26815	0.09677	0.00000							
Lechonia	0.76087*	0.71914*	0.79183**	0.65975*	0.09213	0.70272*	0.66503*	0.75169*	0.72892*	0.44509*	0.30423	0.14806	0.00000						
Korinnos	0.79734**	0.78322**	0.81334**	0.73574**	0.10225*	0.77562*	0.77851*	0.79721**	0.80582**	0.63542**	-0.01548	0.22190*	0.30122*	0.00000					
Pilaia	0.83518**	0.81494*	0.86252**	0.75870*	0.14584*	0.81998*	0.81679*	0.84819*	0.84875*	0.63868*	-0.05882	0.20151	0.29674	-0.01369	0.00000				
Vourvourou	0.83795*	0.81569*	0.87448**	0.73937*	0.08516	0.83227*	0.82663	0.86670*	0.87389*	0.61258*	0.06383	0.15842	0.24892	0.05475	0.03594	0.00000			
P. Koufo	0.76319**	0.75739*	0.78101**	0.67958*	0.31958*	0.74840*	0.72309*	0.79278**	0.74916**	0.26904*	0.68136*	0.23549*	0.48014*	0.63215**	0.65885*	0.65295*	0.00000		
Vassova	0.70100**	0.65435*	0.72786**	0.59878*	-0.06729	0.62373*	0.59322*	0.67939*	0.67906*	0.39698*	0.16763	0.07239	0.03657	0.12592*	0.19252*	0.15551	0.42517*	0.00000	
Drana	0.81715**	0.80478*	0.84467**	0.73664**	0.02761	0.81041*	0.81023*	0.83623*	0.85018*	0.63581*	0.21318	0.23513*	0.34870*	0.06192	0.22837*	0.19123	0.65539**	0.06792	0.00000

Statistical significant differences, *P <0.05 and **P <0.001.
Στατιστικά σημαντικές διαφορές, *P <0.05 και **P <0.001.

Table 3.5. Grouping of the 19 *S. abaster* sampling stations along the sublittoral zone of Greece in 13 regions, based on non-significance of pairwise values of the fixation indices (see Table 3.4).
Πίνακας 3.5. Ομαδοποίηση των 19 σταθμών δειγματοληψίας του είδους *S. abaster*, κατά μήκος της ελληνικής υποπαραλιακής ζώνης σε 13 περιοχές, με βάση τις μη στατιστικά σημαντικές τιμές F_{ST} ανά ζεύγος πληθυσμών (βλ. Πίνακα 3.4).

Sea	Geographical region	Sampling stations	Grouped sampling stations/ Regions
Ionian Sea	Port of Igoumenitsa	Drepano	Ionian Sea (Drepano)
	Amvrakikos Gulf	Neochori	Ionian Sea, Amvrakikos Gulf (Neochori)
	Mitikas	Mitikas	Ionian Sea (Mitikas)
	Tourlida	Tourlida	Ionian Sea (Tourlida)
	Peloponnesus	Kalogria	Ionian Sea, Peloponnesus (Kalogria)
		Katakolo	Ionian Sea, Peloponnesus (Katakolo-Kotichi)
		Kotichi	
		Kaiafa	Ionian Sea, Peloponnesus (Kaiafa)
Aegean Sea	Korinthiakos Gulf	Kechries	Korinthiakos Gulf (Kechries)
	Peloponnesus	Moustos	Aegean Sea, Peloponnesus (Moustos)
	Evoikos Gulf	Livanata	Aegean Sea, Evoikos-Pagasitikos Gulf (Livanata- Karavomilos-Lechonia)
		Karavomilos	
	Pagasitikos Gulf	Lechonia	
	Thermaikos Gulf	Korinnos	Aegean Sea, Thermaikos-Kassandras Gulf (Korinnos-Pilaia- Vourvourou)
		Pilaia	
	Chalkidiki	Vourvourou	
		Porto Koufo	Aegean Sea, Singitikos Gulf (P. Koufo)
	E. Macedonia	Vassova	Aegean Sea, E. Macedonia-Thrace (Vassova- Drana)
	Thrace	Drana	

The 13 sampling regions extracted from the AMOVA analysis (Figure 3.7, Tables 3.4, 3.5) were used in the phylogenetic analysis. The model of sequence evolution that best fitted the 78 haplotypes of the Greek *S. abaster* species was the Maximum Likelihood (ML) HKY+I+G phylogenetic tree (Hasegawa, Kishino and Yano model with gamma distributed rate variation and a proportion of invariable sites,-lnL = 2138.2029; Ti/Tv ratio = 3.3155; F(A) = 0.2962, F(C) = 0.2251, F(G) = 0.1647, F(T) = 0.3140; gamma = 0.8170; BIC = 5310.1118; Hasegawa et al. 1985). These haplotypes formed six distinct clades in the ML tree, most of which were supported with high bootstrap values (Figure 3.8). Three clades belonged to North-Central Aegean region (mtAEG-I, mtAEG-II and mtAEG-III), one in the South Aegean (mtMOU), one in the Korinthiakos Gulf (mtKOR) and one in the Ionian Sea (mtION). The most proliferate clade was the mtAEG-I (38 haplotypes) and the least the mtKOR (2 haplotypes). The exact number of the haplotypes consisting each clade is shown in Figure 3.8.

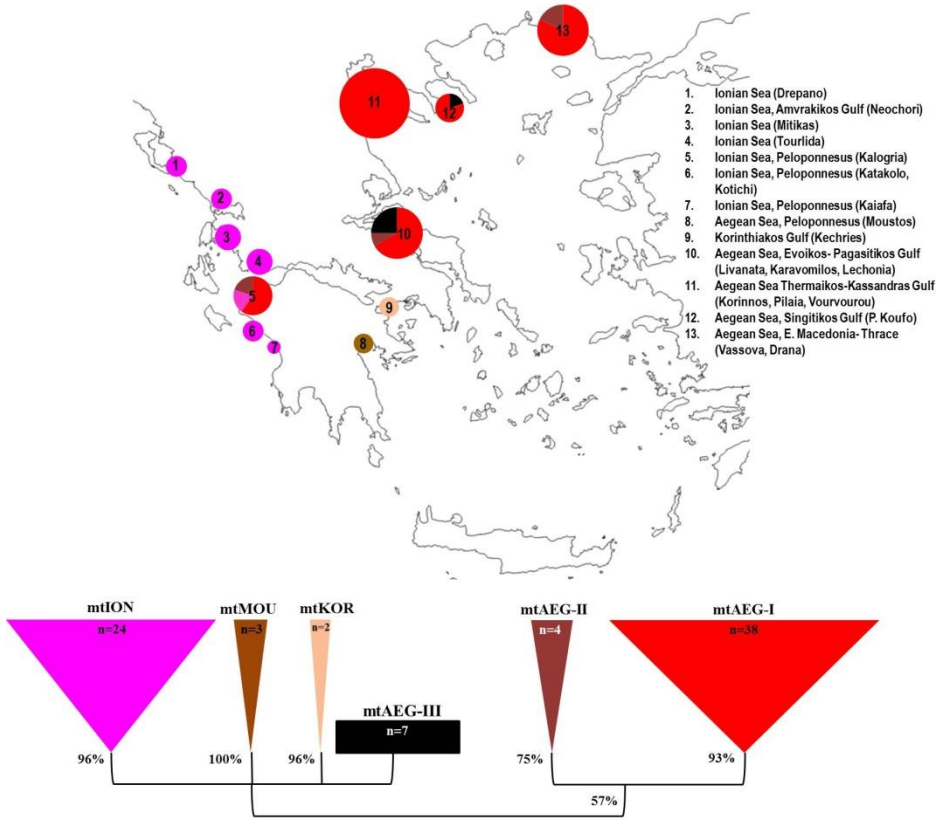


Figure 3.8. Map of Greece and ML phylogenetic tree depicting the relationships between *S. abaster* clades based on the mtDNA Control Region analysis. Circle sizes are proportional to the frequency of haplotypes. The numbers below the clades represent posterior probability support values (*n*, is the number of haplotypes belonging to each clade).

Εικόνα 3.8. Χάρτης της Ελλάδας και φυλογενετικό δέντρο μέγιστης πιθανοφάνειας, όπου απεικονίζονται οι σχέσεις μεταξύ των αλληλουχιών του είδους *S. abaster*, σύμφωνα με τα αποτελέσματα της ανάλυσης της περιοχής ελέγχου του μιτοχονδριακού DNA. Το μέγεθος των κύκλων είναι ανάλογο της συχνότητας των απλοτύπων. Οι αριθμοί στους κλάδους του δέντρου αντιπροσωπεύουν τις τιμές ύστερης πιθανότητας (*n*, είναι ο αριθμός των απλοτύπων κάθε κλάδου).

In addition to the phylogenetic tree, network analysis (Figure 3.9) provided further resolution regarding the relationship between the haplotypes of *S. abaster* in the 13 regions revealed by the AMOVA analysis (Figure 3.7, Tables 3.4, 3.5). Both analyses reinforced the evidence for population of *S. abaster* specimens between the Aegean and the Ionian Sea, with most individuals forming well-delineated clusters of haplotypes. The six defined clades revealed by the phylogenetic tree were also evident in the network analysis. The right part of the network (consisting of 38 haplotypes) corresponded to the mtAEG-I clade. The majority of this group's haplotypes belonged to the Thermaikos- Kassandras Gulf region and were mainly region-specific. Region specificity was also evident for mtMOU and mtKOR clades. Among the individuals of the Ionian Sea the most distinct haplotypes were found in the Neochori region; all being region specific. The haplotypes of the Mitikas region were also region-specific but not as distinct as those from the Neochori region (Figure 3.9).

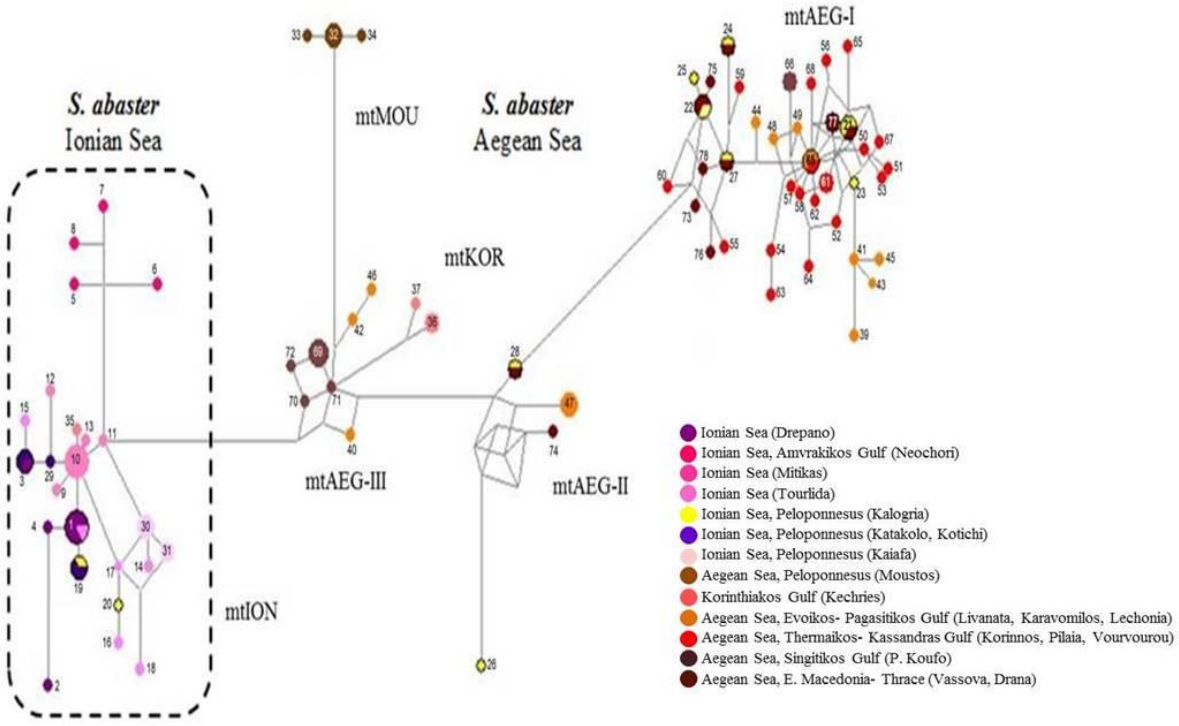


Figure 3.9. Median-joining network of haplotypes of *S. abaster* Greek individuals, based on the analysis of mitochondrial Control Region. Circle sizes are proportional to haplotype frequencies (for the numbering of haplotypes see Table 3.4 in Appendix)

Εικόνα 3.9. Δίκτυο median-joining των σχέσεων των απλοτύπων του είδους *S. abaster* στον ελλαδικό χώρο, σύμφωνα με τα αποτελέσματα της ανάλυσης της περιοχής ελέγχου του μιτοχονδριακού DNA. Το μέγεθος των κύκλων είναι ανάλογο της συχνότητας κάθε απλοτύπου (η αρίθμηση των απλοτύπων είναι σύμφωνα με τον Πίνακα 3.4 του Παραρτήματος).

3.3.1.2. nDNA Locus A1

All PHASE probabilities for phases of individual positions were 0.63 or higher indicating an acceptable accuracy of the analysis (Harrigan et al. 2008).

The nuclear DNA locus A1 of Greek *S. abaster* species showed 21 variable sites (Figure 3.10, Table 3.2.b in Appendix). The most polymorphic individuals (9 polymorphic sites) were found in Kalogria station. The level of nucleotide diversity for the entire sample was π =0.126± 0.008 (ranging from 0 in Mitikas, Moustos, Kechries and Vassova to 0.094 in Kalogria stations), while the haplotype diversity was h= 0.833± 0.024 (ranging from 0 in Mitikas, Moustos, Kechries and Vassova to 0.833 in Korinnos stations) (Table 3.6). Among the 47 analyzed individuals 26 were homozygotic and 21 were heterozygotic (Table 3.6).

GC**G**AGCACATGAAAATGGAAGTTGAGCATCG**A**ATGCGGCGGCAG**C**TGAGC	50
GGGCGCCCGCGAGA**G**GCCAAATCAAGACCGCCTGTAGTGTCTGGGCCAAG	100
GGAC**G**TGTTCCTAAACGTA**G**GCCACCAGTTGAAGACGAGTTTACAGGTCA	150
AGCGGATGACG**A**GGCTCACTTAAAATGA**G**TGGAATGG**C**TATTG**TT**CTGTG	200
ACGG**C**GTTGGCGTCAGT**G**GTGGAGGCCTTTTT**C**GAT**G**CTCAT**AC**TAAGGC	250
TTGCGCTTGAAGGCGGGCAGGCAACATGTGGCCGGAGGGCTTTGTG**C**CTG	300
TATGAGCAAAA**A**TTAGCAGCTTGAAGTCATG**A**GCACGATTATGTCTGTAA	350
AGTCATCAATGGTGATGCGACTCGCAAGCTTGC**T**CGGCAGCGCC**T**CCGGT	400
CGCAAACACTTGCTGTGCTGCG**C**ATCTGCCAAATGTGT	438

Figure 3.10. DNA consensus sequence of a 438bp amplified segment of nuclear DNA Locus A1. Variable positions revealed after the analysis of 47 individuals of *S. abaster* species in the present study are bold and underlined.

Εικόνα 3.10. Ακολουθία αναφοράς του ενισχυμένου τμήματος του πυρηνικού τόπου A1 (438 ζ.β.). Οι πολυμορφικές θέσεις που προέκυψαν από την ανάλυση 47 ατόμων του είδους *S. abaster* στον ελλαδικό χώρο κατά τη διάρκεια της παρούσας μελέτης παρουσιάζονται έντονες και υπογραμμισμένες.

A total of 14 haplotypes were detected in a 438 bp fragment sequenced for 47 individuals from 17 sampling stations (Figure 3.11.a, Table 3.3.b in Appendix). The most abundant and widely distributed haplotype was haplotype 6 (found in individuals in Kalogria, Karavomilos, Lechonia, Korinnos, Pilaia, Vourvourou, P. Koufo, Vassova and Drana stations) (Table 3.3.b in Appendix). The number of haplotypes in each station ranged from one (in individuals from Mitikas, Moustos Kechries and Vassova stations) to three in (in Neochori, Kalogria, Lechonia, Porto Koufo and Drana stations). Contrary to mtDNA results, most individuals didn't form well-delineated clusters of region-specific haplotypes. The individuals from stations with at least one region-specific haplotype were few: Neochori, Livanata, Lechonia, Porto Koufo and Drana (Table 3.6 and 3.3.b in Appendix). This was also revealed by the number of unique haplotypes (UH) and the

percentage of the station-specific haplotypes divided by the number of individuals (W). However as in the mtDNA analysis, the motif of lack of shared haplotypes between the Ionian and Aegean Seas individuals (with the exception of Kalogria station) was retained (Table 3.3.b in Appendix).

Table 3.6. Genetic variability observed in the nuclear Locus A1 of Greek *S. abaster* individuals in the present study. (*n*, sample size; n_{ht}, number of heterozygotic individuals; n_{hm}, number of homozygotic individuals; *Hp*, number of haplotypes; *UHp*, station- specific haplotypes; *V*, the percentage of the total number of haplotypes divided by the number of individuals; *W*, the percentage of the station-specific haplotypes divided by the number of individuals; *S*, number of polymorphic sites; *h*, haplotype diversity; *π*, nucleotide diversity) (values in round brackets correspond to standard deviation values).
Πίνακας 3.6. Γενετική ποικιλότητα του πυρηνικού τόπου Α1 των ατόμων του είδους *S. abaster* στον ελλαδικό χώρο, με βάση τη παρούσα μελέτη (*n*, ο αριθμός των ατόμων; $n_{ht,}$ ο αριθμός των ετερόζυγων ατόμων; n_{hm}, ο αριθμός των ομόζυγων ατόμων; *Hp*, οι απλότυποι κάθε σταθμού; *UHp*, οι μοναδικοί απλότυποι κάθε σταθμού; *V*, το ποσοστό συνολικού αριθμού απλοτύπων/αριθμό ατόμων; *W*, το ποσοστό μοναδικών απλοτύπων/αριθμό ατόμων; *S*, ο αριθμός πολυμορφικών θέσεων; *H*, η απλοτυπική ποικιλότητα; *π*, η νουκλεοτιδική ποικιλότητα) (οι τιμές στις παρενθέσεις αντιστοιχούν στην τυπική απόκλιση).

Sampling Station	n	n_{ht}	n_{hm}	Hp	UHp	V	W	S	h (±)	π (±)
1.Drepano	4	1	3	2	0	50.00	0.00	1	0.250 (± 0.180)	0.007 (± 0.010)
2.Neochori	3	1	2	3	1	100.00	33.33	2	0.600 (± 0.215)	0.019 (± 0.019)
3.Mitikas	1	0	1	1	0	100.00	0.00	0	0	0
4.Tourlida	2	1	1	2	0	100.00	0.00	1	0.500 (± 0.265)	0.014 (± 0.017)
5.Kalogria	5	2	3	3	0	60.00	0.00	9	0.622 (± 0.138)	0.094 (± 0.059)
6.Kaiafa	2	1	1	2	0	100.00	0.00	1	0.500(± 0.265)	0.014 (± 0.017)
7.Moustos	3	0	3	1	0	33.33	0.00	0	0	0
8.Kechries	1	0	1	1	0	100.00	0.00	0	0	0
9.Livanata	3	2	1	2	2	66.67	66.67	3	0.533 (± 0.172)	0.044 (± 0.035)
10.Karavomilos	2	1	1	2	0	100.00	0.00	2	0.500 (± 0.265)	0.028 (± 0.028)
11.Lechonia	3	3	0	3	1	100.00	33.33	2	0.733 (± 0.152)	0.024 (± 0.022)
12.Korinnos	2	1	1	3	0	66.67	0.00	3	0.833 (± 0.222)	0.051 (± 0.043)
13.Pilaia	3	1	2	2	0	66.67	0.00	2	0.600 (± 0.129)	0.033 (± 0.028)
14.Vourvourou	4	3	1	2	0	50.00	0.00	1	0.536 (± 0.123)	0.015 (± 0.016)
15.Porto Koufo	3	2	1	4	2	66.67	66.67	3	0.800 (± 0.172)	0.050 (± 0.038)
16.Vassova	3	0	3	1	0	33.33	0.00	0	0	0
17.Drana	3	2	1	3	1	100.00	33.33	3	0.600 (± 0.215)	0.028 (± 0.025)

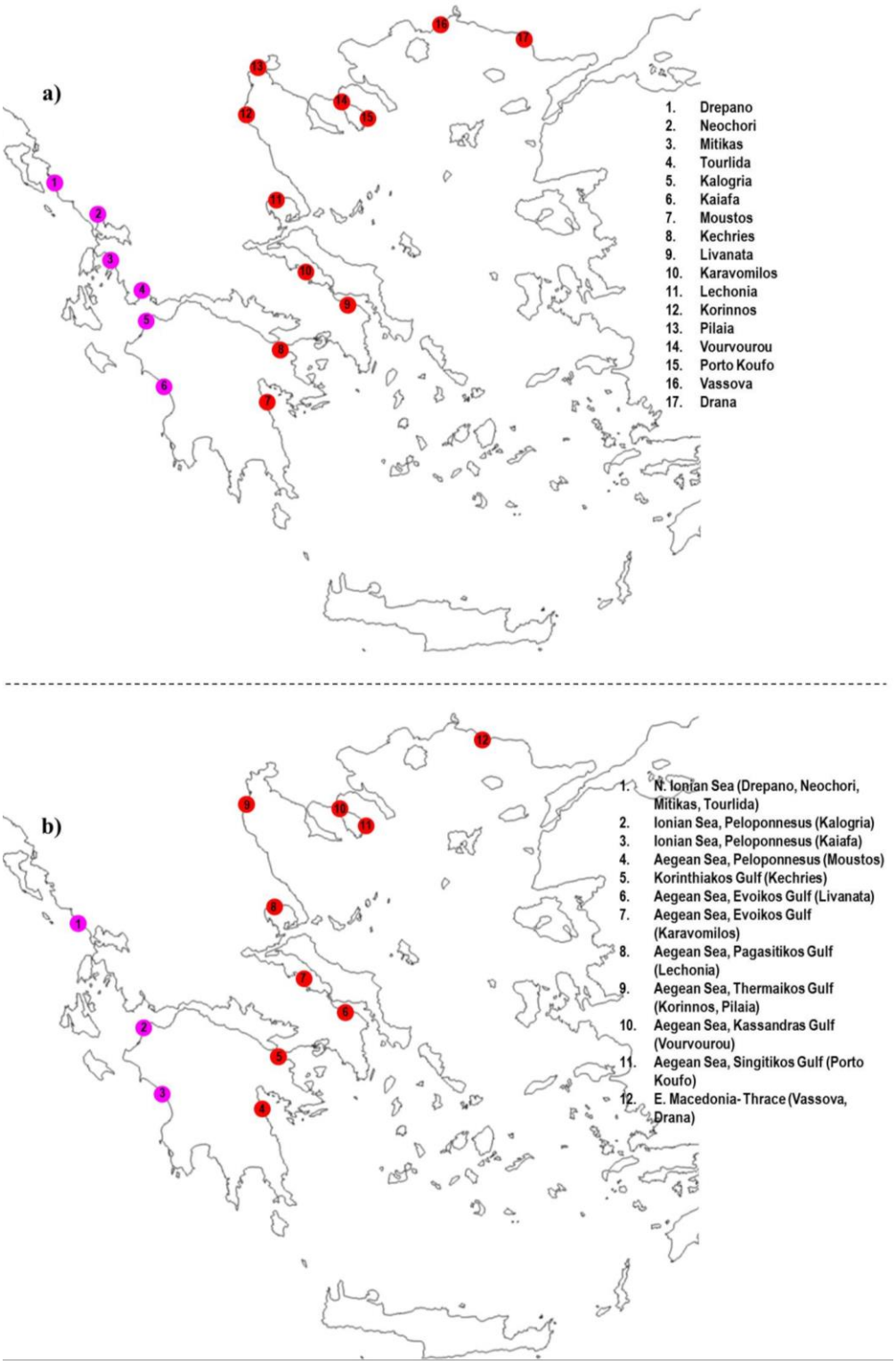


Figure 3.11. Depiction of samples of *S. abaster* species along the sublittoral zone of Greece (a) 17 sampling stations, (b) 12 regions based on non-significance of pairwise values of fixation indices (see Table 3.8).

Εικόνα 3.11. Απεικόνιση των ατόμων του είδους *S. abaster* κατά μήκος της ελληνικής υποπαραλιακής ζώνης σε (a) 17 σταθμούς δειγματοληψίας, και (b) 12 περιοχές, ομαδοποιημένες με βάση τις μη στατιστικά σημαντικές τιμές F_{ST} ανά ζεύγος πληθυσμών (βλ. Πίνακα 3.8).

The results of the AMOVA analysis for the individuals from 17 initial sampling stations resulted in the rejection of the homogeneity hypothesis. The level of differentiation was very high (*Fst* = 0.773; P < 0.001) (Table 3.7.a), i.e. 77.33% of variation was attributed to among-station differences. Subsequently, the 17 sampling stations were grouped in 12 regions according to the results of the pairwise comparison (Tables 3.8, 3.9, Figure 3.11.b). The AMOVA analysis based on this grouping, led to the highest F_{CT} values (F_{CT} = 0.813; P < 0.001) (Table 3.7.b). Therefore, this grouping was highly successful since most of the among-station differences were due to the among-group difference (81.28%) instead of the among-station within groups (-3.08%) (Table 3.7.b). When trying to divide individuals in a simple Aegean / Ionian group scheme, the F_{CT} value was lower (F_{CT} = 0.576; P < 0.05), indicating that even though there was significant distinction between the specimens of the two Seas the grouping of samples into 12 regions was a better depicter of the distribution of genetic variability among *S. abaster* individuals (Table 3.7.c).

Table 3.7. Results of the hierarchical Analysis of Molecular Variance (AMOVA) for *S. abaster* species for the (a) 17 sampling stations; (b) 12 grouped regions; (c) Ionian-Aegean Sea populations based on nuclear locus A1.
Πίνακας 3.7. Αποτελέσματα της ιεραρχικής ανάλυσης μοριακής διακύμανσης (AMOVA) του είδους *S. abaster* για: (a) 17 σταθμούς δειγματοληψίας, (b) 12 ομαδοποιημένες περιοχές, (c) πληθυσμούς Ιονίου-Αιγαίου, με βάση την ανάλυση του πυρηνικού τόπου A1.

Grouping	Source of Variation	Percentage of Variation	Fixation Indices	P values
(a)	Among stations	77.30	F_{ST}= 0.773	P < 0.001
	Within stations	22.70		
(b)	Among groups	81.28	F_{CT} = 0.813	P < 0.001
	Among stations within groups	-3.08	F_{SC} = -0.164	P > 0.05
	Within stations	21.80	F_{ST} = 0.782	P < 0.001
(c)	Among groups	57.61	F_{CT} = 0.576	P < 0.05
	Among stations within groups	26.30	F_{SC} = 0.620	P < 0.001
	Within stations	16.09	F_{ST} = 0.839	P < 0.001

Table 3.8. Values of exact tests for differentiation among the individuals of *S. abaster* from the 17 sampling stations based on nuclear Locus A1.
Πίνακας 3.8. Τιμές του ελέγχου διαφοροποίησης ανά ζεύγος πληθυσμών του είδους *S. abaster* με βάση τον πυρηνικό τόπο A1.

	Drepano	Neochori	Mitikas	Tourlida	Kalogria	Kaiafa	Moustos	Kechries	Livanata	Karavomilos	Lechonia	Korinnos	Pilaia	Vourvourou	P. Koufo	Vassova	Drana
Drepano	0.00000																
Neochori	-0.08244	0.00000															
Mitikas	-0.31765	-0.30435	0.00000														
Tourlida	0.05023	-0.01310	-0.26316	0.00000													
Kalogria	0.69915**	0.65695*	0.56621	0.62746*	0.00000												
Kaiafa	0.61410*	0.44893*	0.52941	0.20000	0.65185*	0.00000											
Moustos	0.98404**	0.96429*	1.00000*	0.97964*	0.35411*	0.98069*	0.00000										
Kechries	0.97613*	0.94106*	1.00000	0.95966	0.15743	0.96172	0.00000	0.00000									
Livanata	0.88985**	0.85217*	0.82353*	0.84516*	0.53698*	0.85464*	0.87368*	0.79661*	0.00000								
Karavomilos	0.94082**	0.90470*	0.90734	0.90909*	0.07261	0.91429*	0.84516*	0.71084	0.74443*	0.00000							
Lechonia	0.94097**	0.91321*	0.91622*	0.91694*	0.10404	0.92141*	0.71111*	0.55429	0.78857*	0.53927*	0.00000						
Korinnos	0.91123**	0.86998*	0.83673	0.86275*	-0.02784	0.87037*	0.67367*	0.43226	0.69677*	-0.13333	0.22750	0.00000					
Pilaia	0.91994**	0.88800*	0.87755*	0.88711*	0.01266	0.89352*	0.70000*	0.53846	0.73750*	-0.10769	0.31111	-0.19740	0.00000				
Vourvourou	0.95378**	0.93215**	0.94461*	0.93916*	0.15130*	0.94250*	0.77798**	0.67957*	0.82650**	0.62791*	0.07711	0.27004	0.39122*	0.00000			
P. Koufo	0.89700**	0.86296*	0.83178*	0.85446*	0.16466	0.86211*	0.70000*	0.53846*	0.71667*	0.04000	0.46667*	0.05306	0.10000	0.53768*	0.00000		
Vassova	0.98208**	0.96000*	1.00000*	0.97716*	0.08065	0.97848*	1.00000**	1.00000*	0.85000*	0.73626*	0.13333	0.37882*	0.40000	0.23323	0.55000*	0.00000	
Drana	0.93179**	0.90196*	0.89967*	0.90391*	0.01025	0.90928*	0.66667*	0.49153*	0.76364*	0.33333	0.06667	0.02890	0.05714	0.13890	0.31892*	0.00000	0.00000

Statistical significant differences, *P <0.05 and **P <0.001.
Στατιστικά σημαντικές διαφορές, *P <0.05 και **P <0.001.

Table 3.9. Grouping of the 17 *S. abaster* sampling stations along the sublittoral zone of Greece in 12 regions, based on non-significance of pairwise values of fixation indices (see Table 3.8).
Πίνακας 3.9. Ομαδοποίηση των 17 σταθμών δειγματοληψίας του είδους *S. abaster*, κατά μήκος της ελληνικής υποπαραλιακής ζώνης σε 12 περιοχές, με βάση τις μη στατιστικά σημαντικές τιμές F_{ST} ανά ζεύγος πληθυσμών (βλ. Πίνακα 3.8).

Sea	Geographical region	Sampling stations	Grouped sampling stations/ Regions
Ionian Sea	Port of Igoumenitsa	Drepano	N. Ionian Sea (Drepano, Neochori, Mitikas, Tourlida)
	Amvrakikos Gulf	Neochori	
	Mitikas	Mitikas	
	Tourlida	Tourlida	
	Peloponnesus	Kalogria	Ionian Sea, Peloponnesus (Kalogria)
		Kaiafa	Ionian Sea, Peloponnesus (Kaiafa)
Aegean Sea	Korinthiakos Gulf	Kechries	Korinthiakos Gulf (Kechries)
	Peloponnesus	Moustos	Aegean Sea, Peloponnesus (Moustos)
	Evoikos Gulf	Livanata	Aegean Sea, Evoikos Gulf (Livanata)
		Karavomilos	Aegean Sea, Evoikos Gulf (Karavomilos)
	Pagasitikos Gulf	Lechonia	Aegean Sea, Pagasitikos Gulf (Lechonia)
	Thermaikos Gulf	Korinnos	Aegean Sea, Thermaikos Gulf (Korinnos-Pilaia)
		Pilaia	
	Chalkidiki	Vourvourou	Aegean Sea, Kassandras Gulf (Vourvourou)
		Porto Koufo	Aegean Sea, Singitikos Gulf (P. Koufo)
	E. Macedonia	Vassova	Aegean Sea, E. Macedonia- Thrace (Vassova-Drana)
	Thrace	Drana	

The 12 sampling regions extracted from the AMOVA analysis were used in the phylogenetic analysis (Figure 3.12). The model of sequence evolution that best fitted the 14 haplotypes of the Greek *S. abaster* species was the Maximum Likelihood (ML) tree K80 (Kimura 2-parameter model (Kimura 1980); -lnL = 160.8965; Ti/Tv ratio = 1.2818; BIC= 418.5481). These haplotypes formed four distinct clades in the ML tree (Figure 3.12). Most clades were supported with high bootstrap values. Three clades belonged to the Aegean region (nAEG-I, nAEG-II and nAEG-III) and one in the Ionian Sea (nION). The most proliferate clade was the nAEG-I (6 haplotypes) and the least the nAEG-II and nAEG-III (2 haplotypes). There was some similarity between the clades of the mitochondrial and the nuclear phylogenetic tree but not complete identity, especially regarding the depiction of the Ionian Sea. The number of the haplotypes consisting each clade is shown in Figure 3.12.

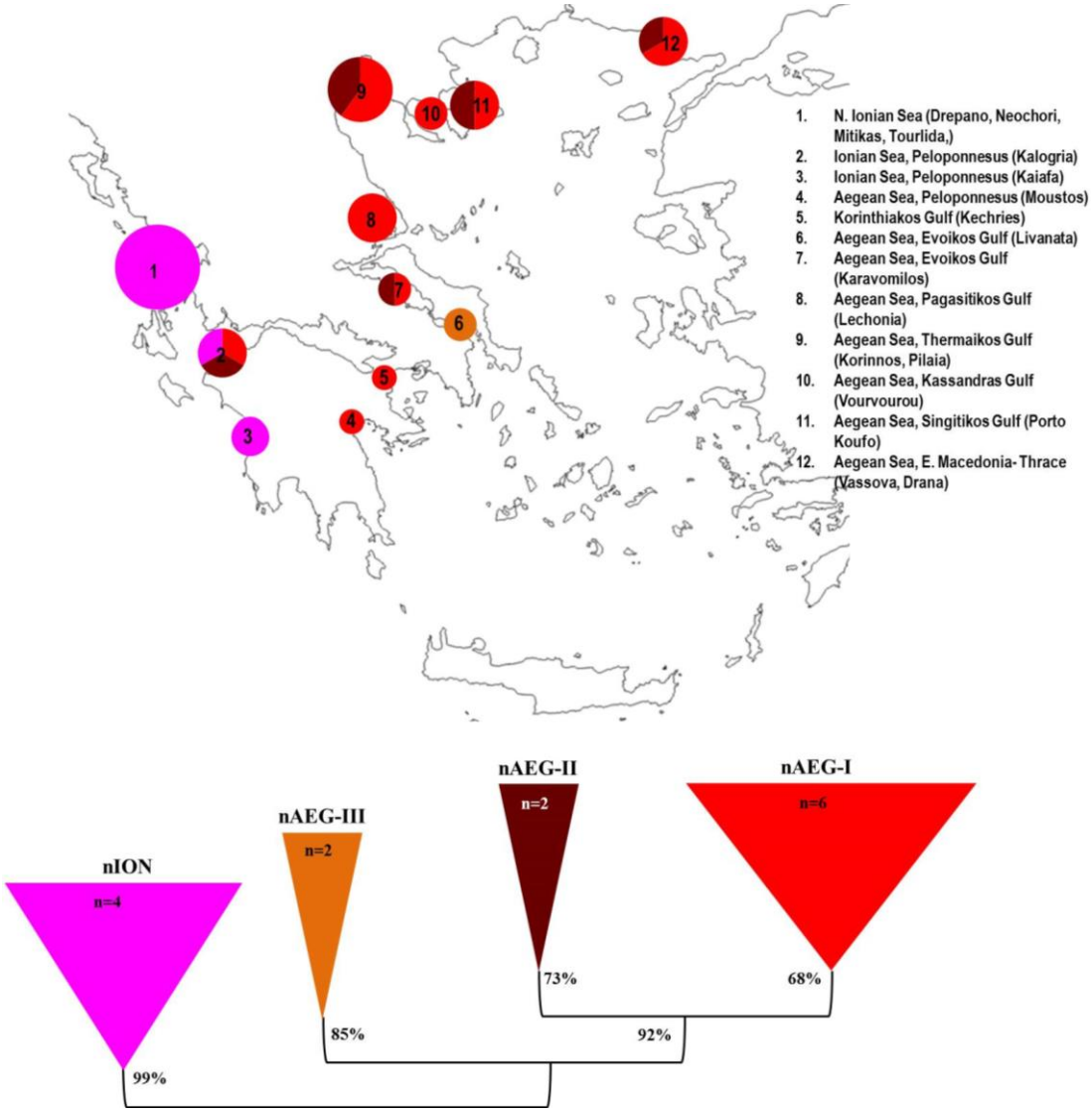


Figure 3.12. Map of Greece and ML phylogenetic tree depicting the relationships between *S. abaster* clades based on the nuclear Locus A1 analysis. Circle sizes are proportional to the frequency of haplotypes. The numbers below the clades represent posterior probability support values (*n*, is the number of haplotypes belonging to each clade).

Εικόνα 3.12. Χάρτης της Ελλάδας και φυλογενετικό δέντρο μέγιστης πιθανοφάνειας, όπου απεικονίζονται οι σχέσεις μεταξύ των ομάδων του είδους *S. abaster*, σύμφωνα με τα αποτελέσματα της ανάλυσης του πυρηνικού τόπου A1. Το μέγεθος των κύκλων είναι ανάλογο των ατόμων κάθε περιοχής και τα χρώματα της συχνότητας των απλοτύπων. Οι αριθμοί στους κλάδους του δέντρου αντιπροσωπεύουν τις τιμές ύστερης πιθανότητας (*n*, ο αριθμός των απλοτύπων κάθε κλάδου).

In addition to the phylogenetic tree, network analysis provided further resolution regarding the relationships between the haplotypes of *S. abaster* in the 12 regions that were revealed by the AMOVA analysis (Figure 3.13). Both analyses reinforced the evidence for differentiation of *S. abaster* species between the Aegean and the Ionian Sea. The four defined clades revealed by the phylogenetic tree were also evident in the network analysis. Three core haplotypes (i.e. most frequent) were found: haplotypes 1, 5 and 6. Contrary to mtDNA results, most of the haplotypes were not region-specific. Among the individuals of the Aegean Sea the most distinct haplotypes were found in the Evoikos Gulf (Livanata) region; all being region-specific.

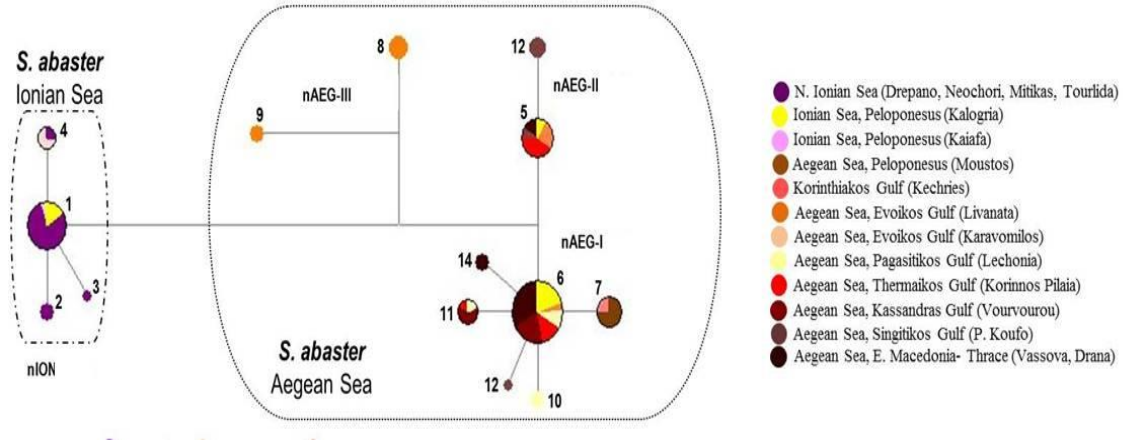


Figure 3.13. Median-joining network of haplotypes of *S. abaster* Greek individuals, based on the analysis of nuclear Locus A1. Circles are proportional to haplotype frequencies (for the numbering of haplotypes see Table 3.3.b in Appendix).

Εικόνα 3.13. Δίκτυο median-joining των σχέσεων των απλοτύπων του είδους *S. abaster* στον ελλαδικό χώρο, σύμφωνα με τα αποτελέσματα της ανάλυσης του πυρηνικού τόπου A1. Το μέγεθος των κύκλων είναι ανάλογο της συχνότητας του κάθε απλοτύπου (η αρίθμηση των απλοτύπων φαίνεται στον Πίνακα 3.3.b του Παρατήματος).

3.3.2. *Syngnathus typhle* species; Greek populations

3.3.2.1. mtDNA Control Region

The mitochondrial DNA control region of Greek individuals of *S. typhle* species showed 22 variable sites (Figure 3.14, Table 3.2.c in Appendix). The level of nucleotide diversity for the entire sample was $\pi = 0.008 \pm 0.002$ (ranging from 0 in individuals from Mitika, Katakolo, Gialova and Vassova stations to 0.010 in Pilaia station), while the haplotype diversity was $h= 0.953 \pm 0.001$ (ranging from 0 in individuals found in Mitika, Katakolo, Gialova and Vassova stations to 1.000 in Katakolo, Gialova, Korinnos, Pilaia and Vassova stations) (Table 3.10).

TTGA**G**TTATTTAAACCACAGAACTAAGACATTTCACTAAG**A**T**A**TGCATAA 50
ACCATTAATGCAAACTACTAAATCTTAGTTTAGTGTTA**C**TGGTTCCTAA**A** 100
A**T**CTAACACAA**C**ACTCATA**A**GTTAAGTTATACCA**C**GACTCCAAAATCGAT 150
TAAATTAAGTAT**C**TTAATGTAGTAAGAGCCTACCTACCAGTCCATTTCTT 200
AATGCCAACGGTTATTGATGGTCAGGCGCCCT**T**ATTGTG**A**GGGTAGCTAC 250
CTAAAAGGTGAATTATTCCTGGCATTTGGCTCCTACTTCAGGTCCATTAA 300
TTTATTAACCCTCGCACTTTCATCGACGCTAGCATAAGTTAATGGTGGAA 350
ATCATACGACTCGTTACCCCCCAAGCCGGGCGTTCACTCCACAGGGGCAG 400
CTGGTTCCTTTTTTCGTTTTCCTTTCAATTAGCATCTCA**G**AGTGCACACG 450
GTATTAGAT**G**A**T**AAGGT**T**GAACATTTCCTTGAATGAGTA**T**ATTCGTTTAA 500
TGTTGGAAAGACATTACATAAGAATTGCATATATCTATTACTAAAGCATA 550
ATACCTAAATTTTAGTCCTAATATTTTTAAGATCGCCCCTTCTTGGTTTA 600
ATTCCGACAAACCCCCTACCCCTTACAACCCT**G**ACATGTCCGCCACTCCT 650
GCAAACCCCTAAGAAACAGGA**A**TGTCCCGAGTAAAACTTATTATCTCACT 700
CGTCAATCAACAAATGTCAT**A**TGTATATAGTATTGT**T**AGATTTTCAAAAC 750
ACACCTGTATGCCATATTTAACTCATTAGACTCAAATTACACTAAGACCT 800
GCTCGCTGTTGTAGCT 816

Figure 3.14. DNA consensus sequence of a 816bp amplified segment of mitochondrial Control Region. Variable positions revealed after the analysis of 24 individuals of Greek *S. typhle* population in the present study are bold and underlined.

Εικόνα 3.14. Ακολουθία αναφοράς του ενισχυμένου τμήματος της περιοχής ελέγχου του μιτοχονδριακού DNA (816 ζ.β.). Οι πολυμορφικές θέσεις που προέκυψαν από την ανάλυση 24 ατόμων του είδους *S. typhle* στον ελλαδικό χώρο παρουσιάζονται έντονες και υπογραμμισμένες.

A total of 17 haplotypes were detected in a 816 bp fragment sequenced for 24 individuals from ten sampling stations (Figure 3.15, Table 3.2.c in Appendix). The most abundant and widely spread haplotype was haplotype 7 (found in individuals from Mitikas, Korinnos and Vourvourou stations) (Table 3.3.c in Appendix). The number of haplotypes in each station ranged from one (Mitikas, Katakolo, Gialova and Vassova) to four

(Drepano). The high percentage of the total number of haplotypes divided by the number of individuals observed in most populations (V) revealed that more individuals need to be examined in order to have a clearer view of haplotype frequencies. The highest number of polymorphic sites was observed in individuals from Drepano and Pilaia stations. However, only Drepano haplotypes were all station-specific, (Tables 3.10, 3.3.c in Appendix). In general, with the exception of haplotype 7 (Katakolo, Korinnos and Vourvourou) and haplotype 11 (Korinnos, Pilaia) there was a striking lack of haplotype sharing among the studied stations, though it should be stressed again that this could be due to the low sample size (Table 3.3.c in Appendix). This was also revealed by the number of unique haplotypes (UHp) and the percentage of the station-specific haplotypes divided by the number of individuals (W). There was no apparent differentiation between the Ionian and Aegean Seas in the distribution of haplotypes (Table 3.3.c in Appendix).

Table 3.10. Genetic variability observed in the mitochondrial DNA control region of Greek *S. typhle* individuals as revealed in the present study (*n*, sample size; *Hp*, number of haplotypes; *UHp*, station-specific haplotypes; *V*, the percentage of the total number of haplotypes divided by the number of individuals; *W*, the percentage of the station-specific haplotypes divided by the number of individuals; *S*, number of polymorphic sites; *h*, haplotype diversity; *π*, nucleotide diversity (values in round brackets correspond to standard deviation values).
Πίνακας 3.10. Γενετική ποικιλότητα της περιοχής ελέγχου του μιτοχονδριακού DNA των ατόμων του είδους *S. typhle* στον ελλαδικό χώρο με βάση τη παρούσα μελέτη (*n*, ο αριθμός των ατόμων; *Hp*, οι απλότυποι κάθε σταθμού; *UHp*, οι μοναδικοί απλότυποι κάθε σταθμού; *V*, το ποσοστό συνολικού αριθμού απλοτύπων/αριθμό ατόμων; *W*, το ποσοστό μοναδικών απλοτύπων/αριθμό ατόμων; *S*, αριθμός πολυμορφικών θέσεων; *h*, η απλοτυπική ποικιλότητα; *π*, η νουκλεοτιδική ποικιλότητα) (οι τιμές στις παρενθέσεις αντιστοιχούν στην τυπική απόκλιση).

Sampling Station	n	Hp	UHp	V	W	S	h (±)	π (±)
1.Drepano	4	4	4	100	100	12	1.000 (±0.177)	0.008 (±0.006)
2.Neochori	3	2	2	66.67	66.67	4	0.667 (±0.314)	0.003 (±0.003)
3.Mitikas	2	1	0	50	0	0	0	0
4.Katakolo	1	1	1	100	100	0	1.000	0
5.Gialova	1	1	1	100	100	0	1.000	0
6.Livanata	3	2	2	66.67	66.67	8	0.667 (±0.314)	0.007 (±0.005)
7.Korinnos	3	2	0	66.67	0	8	0.667 (±0.314)	0.007 (±0.005)
8.Pilaia	3	3	2	100	66.67	12	1.000 (±0.272)	0.010 (±0.008)
9.Vourvourou	3	3	2	100	66.67	9	1.000 (±0.272)	0.008 (±0.006)
10.Vassova	1	1	1	100	100	0	1.000	0

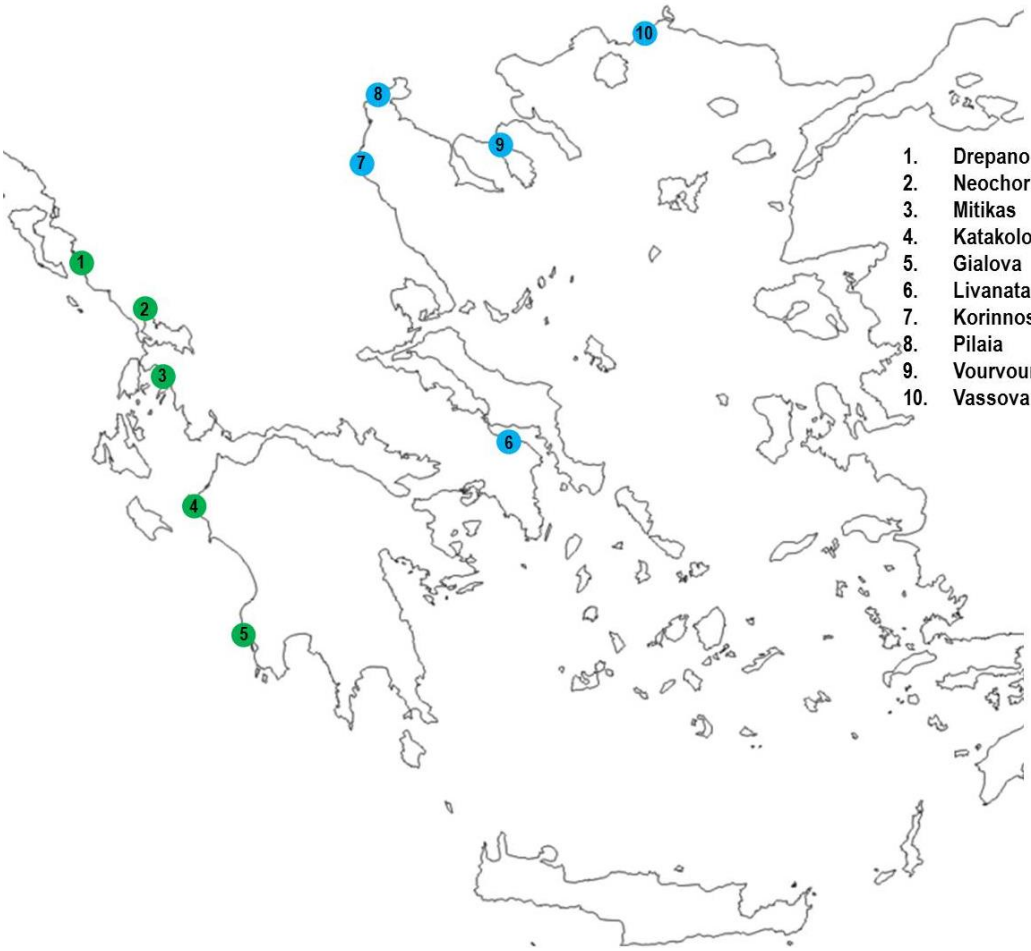


Figure 3.15. Map of sampling sites for *S. typhle* specimens along the sublittoral zone of Greece The collected specimens were used in the mitochondrial control region analysis.
Εικόνα 3.15. Χάρτης στον οποιο απεικόνιζονται οι περιοχές κατά μήκος της ελληνικής υποπαραλιακής ζώνης από τις οποιες συλλέχθησαν τα άτομα του είδους *S. typhle*. Τα άτομα αυτα χρησιμοποιήθηκαν στην ανάλυση της μιτοχονδριακής περιοχής ελέγχου.

The results of the AMOVA analysis among individuals from the 10 sampling stations showed a significant level of differentiation (*Fst* = 0.203; P < 0.05) (Table 3.11.a), i.e. 20.31% of variation was attributed to among-station differences and 79.69% to within-station. When trying to divide individuals in a simple Aegean / Ionian group scheme, the F_{CT} value was zero (F_{CT} = -0.042; P > 0.05), indicating that there is no significant distinction between the specimens of the two Seas (Tables 3.11.b; 3.12).

Table 3.11. Results of the hierarchical analysis of molecular variance (AMOVA) for *S. typhle* species for the: (a) ungrouped 10 sampling station and (b) Ionian-Aegean Sea individuals based on mtDNA control region.
Πίνακας 3.11. Αποτελέσματα της ιεραρχικής ανάλυσης μοριακής διακύμανσης (AMOVA) του είδους *S. typhle* για: (a) 10 σταθμούς δειγματοληψίας και (b) άτομα Ιονίου- Αιγαίου, με βάση την ανάλυση της περιοχής ελέγχου του μιτοχονδριακού DNA.

Grouping	Source of Variation	Percentage of Variation	Fixation Indices	P values
(a)	Among stations	20.31	F_{ST}= 0.203	P < 0.05
	Within stations	79.69		
(b)	Among groups	-4.18	F_{CT} = -0.042	P > 0.05
	Among stations within groups	23.03	F_{SC} = 0221	P < 0.05
	Within stations	81.15	F_{ST} = 0.189	P < 0.05

Table 3.12. Values of exact tests for differentiation among individuals of *S. typhle* from ten sampling stations based on mtDNA Control Region.
Πίνακας 3.12. Τιμές του ελέγχου διαφοροποίησης ανά ζεύγος πληθυσμών του είδους *S. typhle* στην περιοχή ελέγχου του μιτοχονδριακού DNA.

	Drepano	Neochori	Mitikas	Katakolo	Gialova	Livanata	Korinnos	Pilaia	Vourvourou	Vassova
Drepano	0.00000									
Neochori	0.16410	0.00000								
Mitikas	0.33758	0.77251	0.00000							
Katakolo	-0.13043	0.46667	1.00000	0.00000						
Gialova	0.16129	0.63636	1.00000	1.00000	0.00000					
Livanata	0.09159	0.40984	0.57143	0.38462	0.30435	0.00000				
Korinnos	0.13501	0.40984	-0.20000	0.33333	-0.77778	0.31429	0.00000			
Pilaia	-0.03776	0.22581	0.39241	-0.26316	0.00000	0.10448	0.10448	0.00000		
Vourvourou	-0.03138	0.29091	0.07692	-0.12500	-0.20000	0.27143	-0.15909	0.03077	0.00000	
Vassova	-0.13043	0.69231	1.00000	1.00000	1.00000	0.30435	0.30435	-0.50000	0.21739	0.00000

Statistical significant differences, *P <0.05; Στατιστικά σημαντικές διαφορές, *P <0.05.

The model that best fitted the 17 identified haplotypes of *S. typhle* species was the Maximum Likelihood (ML) HKY+I phylogenetic tree (Hasegawa, Kishino and Yano model with invariable sites, Hasegawa et al. 1985; -lnL = 1355.2366; Ti/Tv ratio = 4.0871; F(A) = 0.2993, F(C) = 0.2269, F(G) = 0.1670, F(T) = 0.3068; BIC = 2958.5366). The ML tree revealed that there were no distinct clades formed (Figure 3.16). Network analysis further showed that, even though the haplotypes in their majority were station- specific there was no evidence of distinction between the Ionian and the Aegean Seas (Figure 3.17).

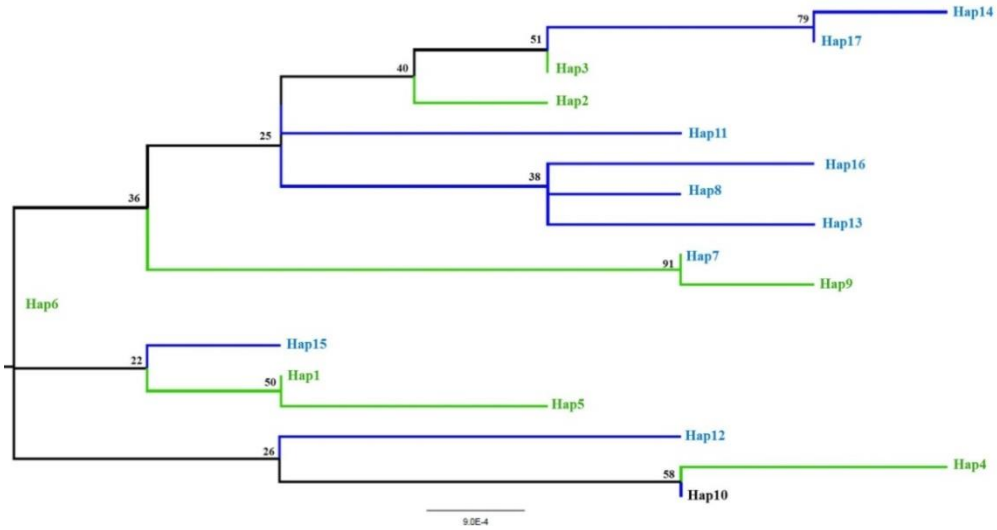


Figure 3.16. Maximum Likelihood HKY+I phylogenetic tree depicting the relationships between Greek haplotypes of *S. typhle* species in the Aegean (—) and the Ionian (—) Sea as inferred from mtDNA Control Region analysis.

Εικόνα 3.16. Φυλογενετικό δέντρο μέγιστης πιθανοφάνειας (μοντέλο HKY+I), όπου απεικονίζονται οι σχέσεις των απλοτύπων του είδους *S. typhle* από το Αιγαίο (—) και το Ιόνιο (—) Πέλαγος, σύμφωνα με τα αποτελέσματα της ανάλυσης του περιοχής ελέγχου του μιτοχονδριακού DNA.

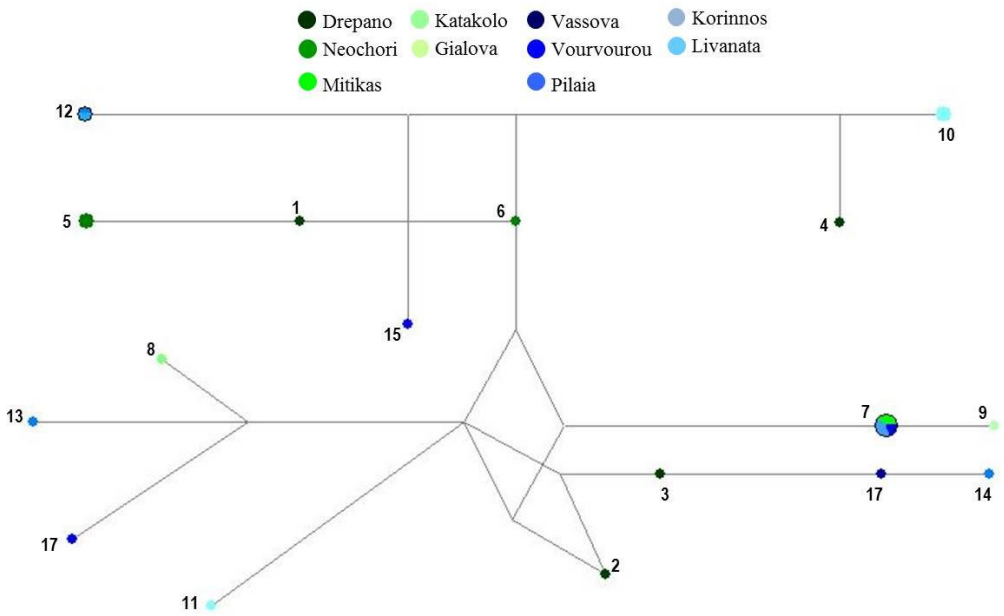


Figure 3.17. Median-joining network of haplotypes of *S. typhle* Greek specimens, based on the analysis of mitochondrial Control Region. Circles are proportional to haplotype frequencies (for the numbering of haplotypes see Table 3.3.c in Appendix).

Εικόνα 3.17. Δίκτυο median-joining των σχέσεων των απλοτύπων του είδους *S. typhle* στον ελλαδικό χώρο, σύμφωνα με τα αποτελέσματα της ανάλυσης της περιοχής ελέγχου του μιτοχονδριακού DNA. Το μέγεθος των κύκλων είναι ανάλογο της συχνότητας του κάθε απλοτύπου (η αρίθμηση των απλοτύπων παρουσιάζεται στον Πίνακα 3.3.c του Παραρτήματος).

3.3.2.2. nDNA Locus A1

The nuclear DNA A1 Locus of Greek individuals of *S. typhle* species included 10 variable sites (Figure 3.18, Table 3.2.d in Appendix). The level of nucleotide diversity for the entire sample was $\pi = 0.036 \pm 0.005$ (ranging from 0 in individuals from Gialova and Vourvourou stations to 0.063 in Neochori stations), while the haplotype diversity was h= 0.689 ± 0.065 (ranging from 0 in Gialova and Vourvourou to 1.000 in Katakolo, Kechries and Vassova stations) (Table 3.13). Among the 26 analyzed individuals 11 were homozygotic and 15 were heterozygotic (Table 3.13).

GCGAGCACATGAAAATGGAAGTTGAGCAT**T**GGATGCGGCGGCAGCTGAGC	50
GGGCACCC**C**CGAGAAGCCAAATCAAGACCGCCTGTAG**T**GTCTGGGCCAAG	100
GGACGTGTTCCTAAACGTAGGCCACCAGTTGAAGACGAGTTTACAGGCCA	150
AGCGGATGACAGGGCTCACTTAAAATGAGTGGAATGGATATTGTTCTGTG	200
ACGGCGTTGGCGTCAGTGGTGGAG**G**CCTTTTTGGATGCTCATTGTAAGGC	250
TTGCGCTTGGAGGCGGGCAGGCAACATGTGGCCG**G**AGGGCTTTGTGTCTG	300
TATGAGCAAAA**A**TTAGCAGCTTGAAGTCATG**A**GCACGATTATGTCTGTAA	350
AGTCATCAATGGTGATGCGACTCGCAAGCTTGCTCGGCAGCGCC**TA**CG**G**T	400
CGCAAACACTTGCTGTGCTGCGCATCTGCCAAATGTGT	438

Figure 3.18. DNA consensus sequence of a 438bp amplified segment of nuclear Locus A1. Variable positions revealed after the analysis of 26 individuals of the *S. typhle* species in the present study are bold and underlined.
Εικόνα 3.18. Ακολουθία αναφοράς του ενισχυμένου τμήματος του πυρηνικού τόπου A1 (438 ζ.β.). Οι πολυμορφικές θέσεις που προέκυψαν από την ανάλυση 26 ατόμων του είδους *S. typhle* στον ελλαδικό χώρο κατά τη διάρκεια της παρούσας μελέτης παρουσιάζονται έντονες και υπογραμμισμένες.

A total of 11 haplotypes were detected in a 438 bp fragment sequenced for 26 individuals from 11 sampling stations (Figure 3.15, Table 3.3.d in Appendix). The most abundant and widely shared haplotype was haplotype 1 (found in individuals from Drepano, Neochori, Mitikas, Katakolo, Livanata, Korinnos, Pilaia Vourvourou and Vassova stations) (Table 3.3.d in Appendix). The number of haplotypes in each station ranged from one (Gialova and Vourvourou) to four (Drepano, Neochori, Livanata and Pilaia) (Tables 3.13 and 3.3.d in Appendix). The high percentage of the total number of haplotypes divided by the number of individuals observed in most stations (V) is an indicator of the species polymorphism; in most cases each individual corresponded to a haplotype. However more individuals need to be examined in order to have a clear view of haplotype frequencies. In general, most of the haplotypes were not station- specific and there was also high level of haplotype sharing among individuals from different stations and among the Ionian and Aegean Seas (Tables 3.13, 3.3.d in Appendix).

Table 3.13. Genetic variability observed in the nuclear Locus A1 of Greek *S. typhle* individuals, as revealed in the present study (*n*, sample size; n_{ht}, number of heterozygotic individuals; n_{hm}, number of homozygotic individuals; *Hp*, number of haplotypes; *UHp*, station-specific haplotypes; *V*, the percentage of the total number of haplotypes divided by the number of individuals; *W*, the percentage of the station-specific haplotypes divided by the number of individuals; *S*, number of polymorphic sites; *h*, haplotype diversity; *π*, nucleotide diversity) (values in round brackets correspond to standard deviation values).
Πίνακας 3.13. Γενετική ποικιλότητα του πυρηνικού τόπου Α1 των ατόμων του είδους *S. typhle* στον ελλαδικό χώρο με βάση την παρούσα μελέτη (*n*, ο αριθμός των ατόμων; $n_{ht,}$ ο αριθμός των ετερόζυγων ατόμων; n_{hm}, ο αριθμός των ομόζυγων ατόμων*; Hp*, οι απλότυποι κάθε σταθμού; *UHp*, οι μοναδικοί απλότυποι κάθε σταθμού; *V*, το ποσοστό συνολικού αριθμού απλοτύπων/αριθμό ατόμων; *W*, το ποσοστό μοναδικών απλοτύπων/αριθμό ατόμων; *S*, αριθμός πολυμορφικών θέσεων; *h*, η απλοτυπική ποικιλότητα; *π,* η νουκλεοτιδική ποικιλότητα) (οι τιμές στις παρενθέσεις αντιστοιχούν στην τυπική απόκλιση).

Sampling Station	n	n_{ht}	n_{hm}	Hp	UHp	V	W	S	h (±)	π (±)
1.Drepano	4	2	2	4	2	100.00	50.00	4	0.643 (±0.184)	0.028 (±0.024)
2.Neochori	3	3	0	4	1	133.33	33.33	5	0.867 (±0.130)	0.063 (±0.046)
3.Mitikas	3	1	2	3	0	100.00	0.00	2	0.733 (±0.155)	0.024 (±0.022)
4.Katakolo	1	1	0	2	0	200.00	0.00	2	1.000 (±0.500)	0.056 (±0.068)
5.Gialova	1	0	1	1	1	100.00	100.00	0	0	0
6.Kechries	1	1	0	2	0	200.00	0.00	2	1.000 (±0.500)	0.056 (±0.068)
7.Livanata	4	2	2	4	1	100.00	25.00	4	0.643 (±0.184)	0.038 (±0.030)
8.Korinnos	2	1	1	2	0	100.00	0.00	2	0.500 (±0.265)	0.028 (±0.028)
9.Pilaia	3	3	0	4	0	133.33	0.00	5	0.867 (±0.130)	0.057 (±0.043)
10.Vourvourou	3	0	3	1	0	33.33	0.00	0	0	0
11.Vassova	1	1	0	2	1	200.00	100.00	1	1.000 (±0.500)	0.028 (±0.040)

Table 3.14. Results of the hierarchical analysis of molecular variance (AMOVA) for *S. typhle* species for the: (a) 11 sampling stations and (b) Ionian-Aegean Sea individuals, based on the nuclear Locus A1.
Πίνακας 3.14. Αποτελέσματα της ιεραρχικής ανάλυσης μοριακής διακύμανσης (AMOVA) του είδους *S. typhle* για: (a) 11 σταθμούς δειγματοληψίας και (b) άτομα Ιονίου- Αιγαίου με βάση τον πυρηνικό τόπο Α1.

Grouping	Source of Variation	Percentage of Variation	Fixation Indices	P values
(a)	Among stations	4.51	F_{ST}= 0.045	P > 0.05
	Within stations	95.49		
(b)	Among groups	-1.11	F_{CT} = -0.011	P > 0.05
	Among stations within groups	5.15	F_{SC} = 0.051	P > 0.05
	Within stations	95.96	F_{ST} = 0.040	P > 0.05

The results of the AMOVA analysis of the individuals from the 11 sampling stations (Figure 3.5) indicated a low, non-significant level of differentiation (*Fst* = 0.045; P > 0.05) (Table 3.14.a), i.e. 4.51% of variation was attributed to among-station differences and 95.49% to within-station. When trying to divide individuals in a simple Aegean / Ionian group scheme, the F_{CT} value was zero (F_{CT} = -0.011; P > 0.05), indicating that there was no significant distinction between the specimens of the two Seas and no genetic differentiation among Greek population of *S. typhle* species (Tables 3.15, 3.14.b).

Table 3.15. Values of the exact tests for differentiation among individuals of *S. typhle* from the eleven sampling stations based on nuclear Locus A1.
Πίνακας 3.15. Τιμές του ελέγχου διαφοροποίησης ανά ζεύγος πληθυσμών του είδους *S. typhle* στον πυρηνικό τόπο A1.

	Drepano	Neochori	Mitikas	Katakolo	Gialova	Kechries	Livanata	Korinnos	Pilaia	Vourvourou	Vassova
Drepano	0.00000										
Neochori	0.03895	0.00000									
Mitikas	-0.06946	-0.04444	0.00000								
Katakolo	0.07097	-0.30435	-0.04587	0.00000							
Gialova	0.47418	0.28671	0.55429	0.50000	0.00000						
Kechries	-0.03597	-0.30435	-0.25275	-0.33333	0.50000	0.00000					
Livanata	0.03297	0.06731	0.06894	0.12589	0.39802	0.05398	0.00000				
Korinnos	-0.06667	-0.12052	-0.11392	-0.37931	0.52941	-0.08108	0.03614	0.00000			
Pilaia	0.02194	-0.14706	-0.05600	-0.36196	0.29811	-0.22652	0.00747	-0.17647	0.00000		
Vourvourou	-0.04025	0.15000	0.13333	0.53846	1.00000	0.53846	0.04901	0.11111	0.11429	0.00000	
Vassova	0.00000	-0.02304	0.09434	0.00000	0.66667	0.00000	-0.10891	0.00000	-0.04082	0.53846	0.00000

Statistical significant differences, *P <0.05.
Στατιστικά σημαντικές διαφορές, *P <0.05.

The model of sequence evolution that best fitted the 11 identified haplotypes of *S. typhle* species was the Maximum Likelihood (ML) JC phylogenetic tree (Jukes-Cantor (Jukes and Cantor 1969); -lnL = 115.0944; BIC = 301.8591). The ML tree revealed that there were no distinct clades formed (Figure 3.19). Network analysis further showed that, most haplotypes were not station- specific and that there was no evidence of distinction between the Ionian and the Aegean Seas. Instead there is a core haplotype (i.e. most frequent) -1- which was surrounded by the rest of the haplotypes by a star-like pattern (Figure 3.20).

The results of the nDNA analysis, network and phylogenetic tree analyses as well as the AMOVA analysis supported the panmixia of the Greek population of *S. typhle* species. This result is opposed to the well-delineated Ionian and Aegean clusters of haplotypes of *S. abaster* species.

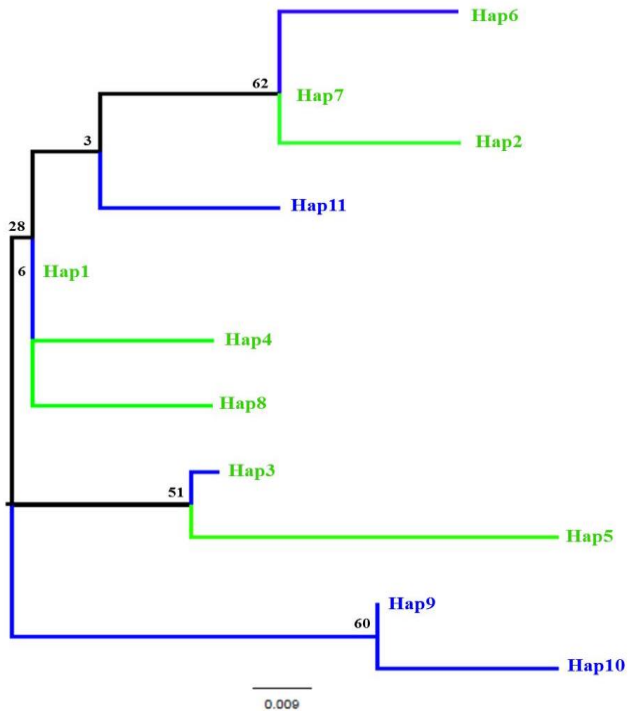


Figure 3.19. Haplotype Maximum Likelihood JC phylogenetic tree depicting the relationships between haplotypes of *S. typhle* species in the Aegean (▬) and the Ionian (▬) Sea as inferred from the nuclear Locus A1.

Εικόνα 3.19. Φυλογενετικό δέντρο μέγιστης πιθανοφάνειας (μοντέλο JC), όπου απεικονίζονται οι σχέσεις των απλοτύπων του είδους *S. typhle* από το Αιγαίο (▬) και το Ιόνιο (▬) Πέλαγος, σύμφωνα με τα αποτελέσματα της ανάλυσης της περιοχής ελέγχου του πυρηνικού τόπου A1.

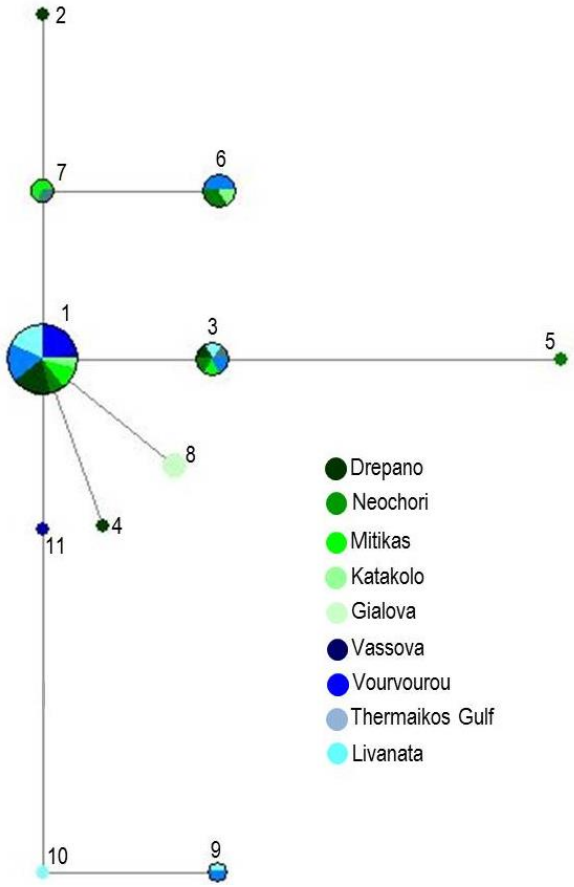


Figure 3.20. Median-joining network of haplotypes of *S. typhle* Greek specimens, based on the analysis of nuclear Locus A1. Circles are proportional to haplotype frequencies.

Εικόνα 3.20. Δίκτυο median-joining των σχέσεων απλοτύπων του είδους *S. typhle* στον ελλαδικό χώρο, σύμφωνα με τα αποτελέσματα της ανάλυσης του πυρηνικού τόπου A1. Το μέγεθος των κύκλων είναι ανάλογο της συχνότητας του κάθε απλοτύπου, της παρούσας μελέτης.

3.3.3. *Syngnathus typhle* species; European population

3.3.3.1. mtDNA Control Region

A total of 111 haplotypes were detected in a 816 bp fragment sequenced for 356 individuals from thirteen sampling stations (Figures 3.21, 3.22). The European haplotypes (Hap 1-95, GenBank accession number: HM773035- HM7 73140) were described in detail by Wilson and Veraguth (2010). The majority of the Greek haplotypes (Hap 96-111) were region specific (Figure 3.22) and no available data existed so far. They displayed a closer affinity with the haplotypes of the Marmara Sea but there was also a shared haplotype with the Black Sea (haplotype 75) and two more intermediate between Marmara and Black Sea (haplotypes 96, 106) (Figure 3.22). There was a striking lack of haplotype sharing between W. Mediterranean Sea and Greek specimens. The Greek and Adriatic Seas samples formed distinct clusters, too. However, a single individual from VEN carried a haplotype (haplotype 61) distinct from the other Adriatic individuals and closely related to the Greek haplotypes (Figure 3.22).

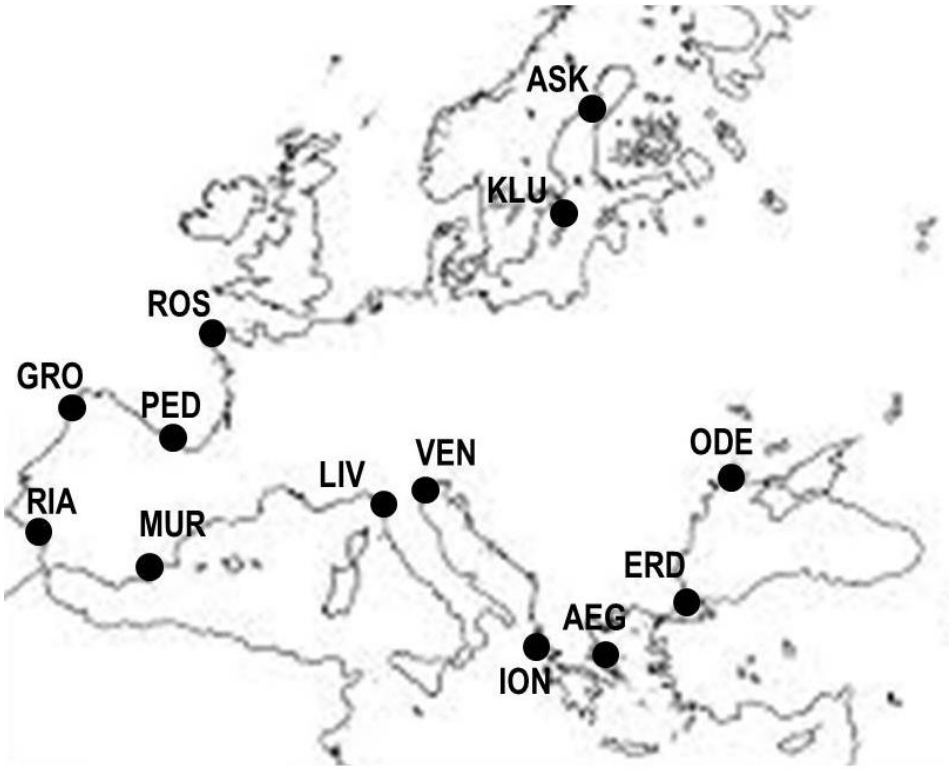


Figure 3.21. Map of the sites that *S. typhle* specimens were collected along the sublittoral zone of the European coastline. The Greek samples (ION and AEG) were collected in the present study. The rest of the European sequences were retrieved from GenBank (Wilson and Veraguth 2010).
Εικόνα 3.21. Απεικόνιση των πληθυσμών του είδους *S. typhle* κατά μήκος της ευρωπαϊκής υποπαραλιακής ζώνης. Τα δείγματα από τον ελλαδικό χώρο (ION και AEG) συλλέχτηκαν στη παρούσα μελέτη. Για τους πληθυσμούς των υπόλοιπων ευρωπαϊκων περιοχων χρησιμοποιήθηκαν οι κατατεθημένες αλληλουχίες από τους Wilson and Veraguth (2010) στη βάση Genbank.

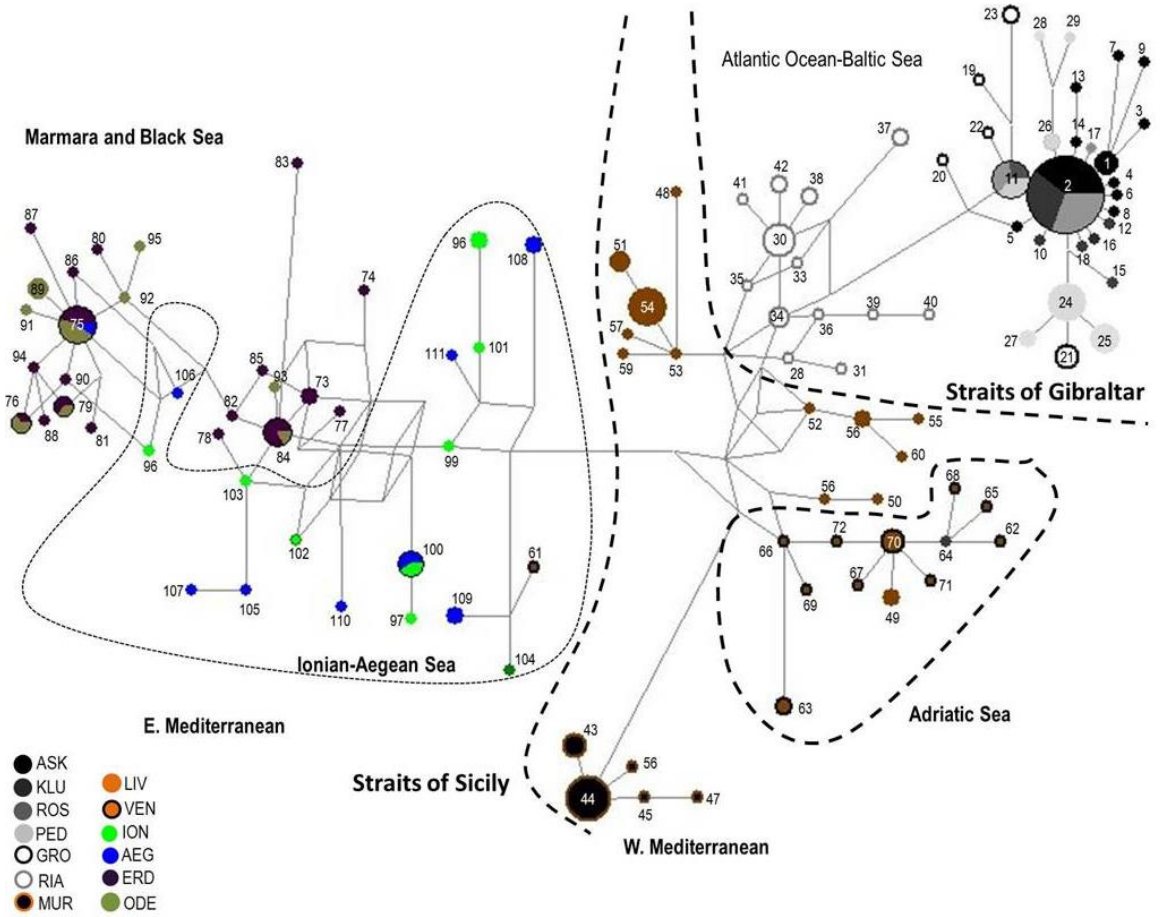


Figure 3.22. Median-joining network of haplotypes of *S. typhle* European populations, based on the analysis of mitochondrial Control Region. Circles are proportional to haplotype frequencies.
Εικόνα 3.22. Δίκτυο median-joining των σχέσεων των απλοτύπων των πληθυσμών του είδους *S. typhle* στον ευρωπαϊκό χώρο, σύμφωνα με τα αποτελέσματα της ανάλυσης της περιοχής ελέγχου του μιτοχονδριακού DNA. Το μέγεθος κύκλων είναι ανάλογο της συχνότητας του κάθε απλοτύπου.

3.3.3.2. nDNA Locus A1

A total of 35 haplotypes were detected in a 438 bp fragment sequenced for 216 individuals from thirteen sampling stations (Figure 3.23). Haplotypes 1-4, 7-22, 24-25, 27-35 correspond to the European haplotypes (GenBank accession number: HM773141–HM773173) which were described in detail by Wilson and Veraguth (2010). Haplotypes 5, 6, 23 and 26 were described for the first time in the present study. The most abundant and widely distributed haplotype was haplotype 3 (Figure 3.23). In general, there was a striking lack of haplotype structure with many shared haplotypes across the samples. Greek population shared haplotypes with individuals from all other studied regions (Figure 3.23). However, the majority of Greek haplotypes belonged to haplotype 4 (Figure 3.23). This haplotype was also common within individuals form the Western Mediterranean and the Atlantic Ocean. Even though there were two core haplotypes (3 and 32) only a few individuals of Greek *S. typhle* species possessed haplotype 3 (Figure 3.23). The difference between haplotypes 3 and 4 is attributed to one base substitution in position 332. In this position the consensus sequence has an Adenine (A), whereas haplotype 4 has a Thymine (T).

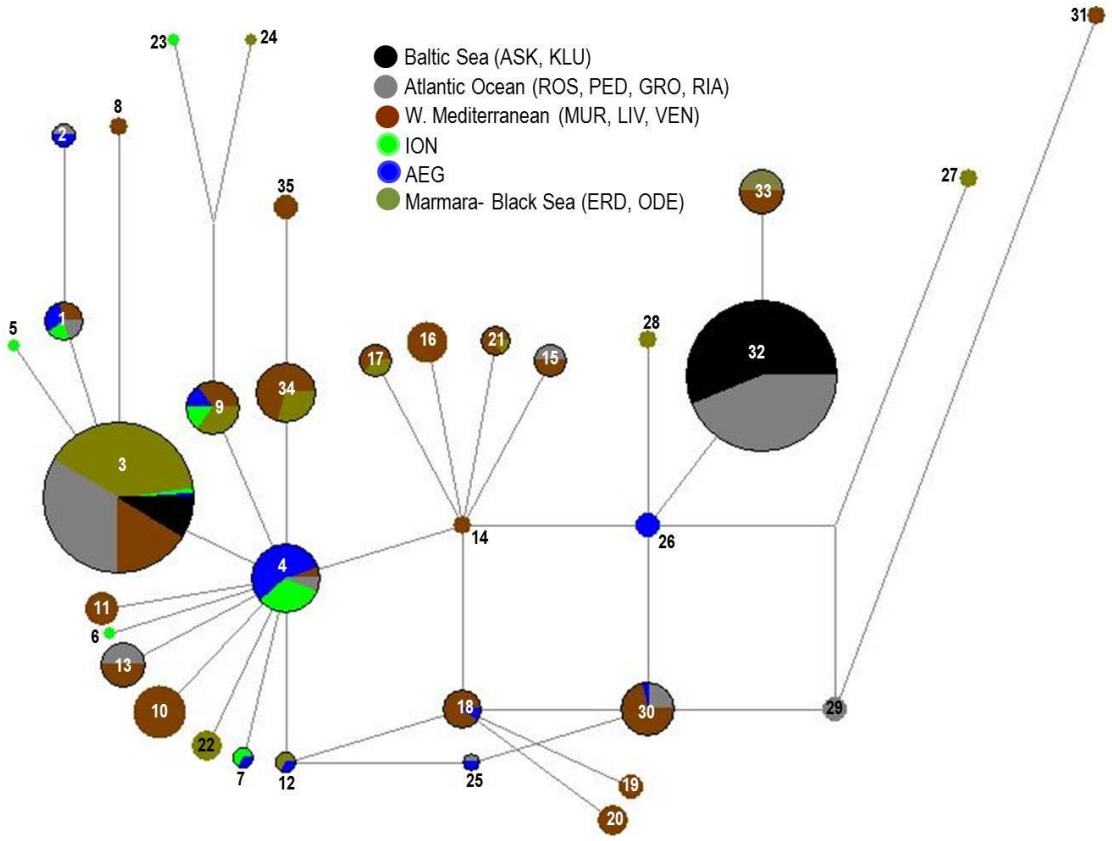


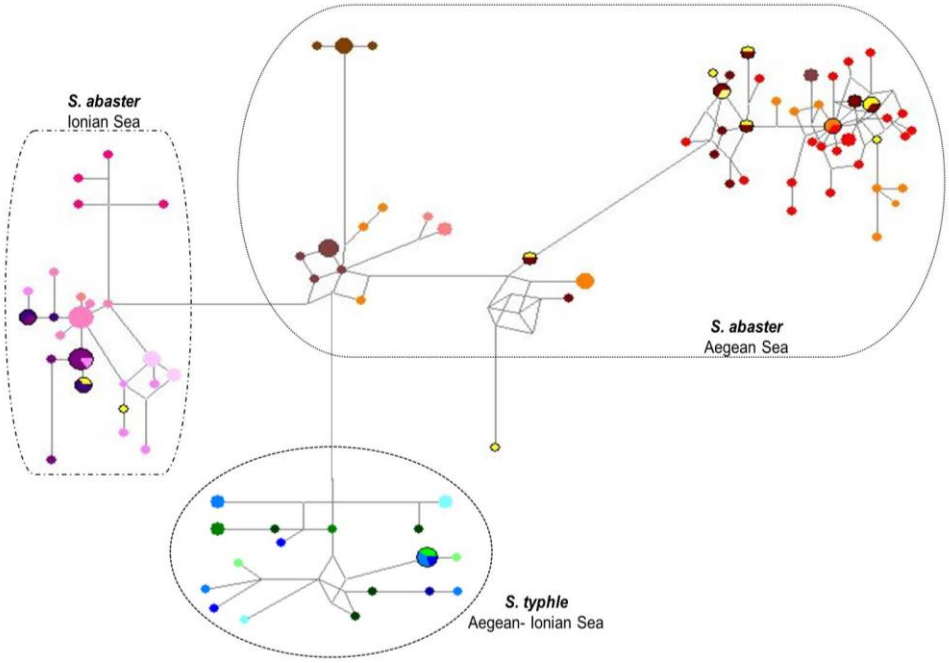
Figure 3.23. Median-joining network of haplotypes of *S. typhle* European haplotypes, based on the analysis of nuclear Locus A1. Circles are proportional to haplotype frequencies.
Εικόνα 3.23. Δίκτυο median-joining των σχέσεων των απλοτύπων του είδους *S. typhle* στον ευρωπαϊκό χώρο, σύμφωνα με τα αποτελέσματα της ανάλυσης του πυρηνικού τόπου A1. Το μέγεθος των κύκλων είναι ανάλογο της συχνότητας του κάθε απλοτύπου.

3.3.4. Phylogenetic relationships between *Syngnathus abaster* and *Syngnathus typhle* species

According to the phylogenetic analysis of the Greek samples based on the Control Region of the mtDNA, *S. abaster* and *S. typhle* formed monophyletic groups (Figure 3.24.a). A total of 95 haplotypes were detected in a 816 bp fragment sequenced for 139 individuals. No shared haplotypes were found between *S. typhle* and *S. abaster* species, excluding the phenomenon of hybridization. The same results were also inferred from the phylogenetic analysis based on the Locus A1 of the nDNA (Figure 3.24.b).

When the Greek haplotypes of *S. abaster* were analyzed with all of the European haplotypes of *S. typhle*, the relative position of the two species revealed by the Greek populations did not alter (Figure 3.25). Both markers revealed that the two species formed two monophyletic groups (Figure 3.25.)

a)



b)

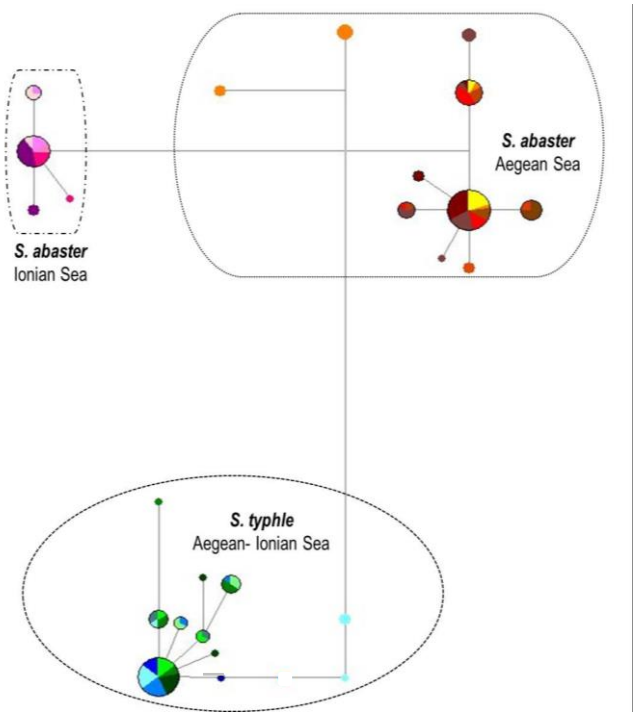


Figure 3.24. Median-joining network of haplotypes of *S. abaster* and *S. typhle* Greek populations, based on the analysis of a) mitochondrial Control Region and b) nuclear Locus A1. Circles are proportional to haplotype frequencies. Circles' colors are after Figures 3.9, 3.13, 3.16, 3.20.
Εικόνα 3.24. Δίκτυο median-joining των σχέσεων των απλοτύπων των πληθυσμών των ειδών *S. abaster* και *S. typhle* στον ελλαδικό χώρο, σύμφωνα με τα αποτελέσματα της ανάλυσης a) της περιοχής ελέγχου του μιτοχονδριακού DNA και b) του πυρηνικού τόπου A1. Το μέγεθος των κύκλων είναι ανάλογο της συχνότητας του κάθε απλοτύπου. Τα χρώματα αντιστοιχούν σε αυτά από τις Εικόνες 3.9, 3.13, 3.16, 3.20.

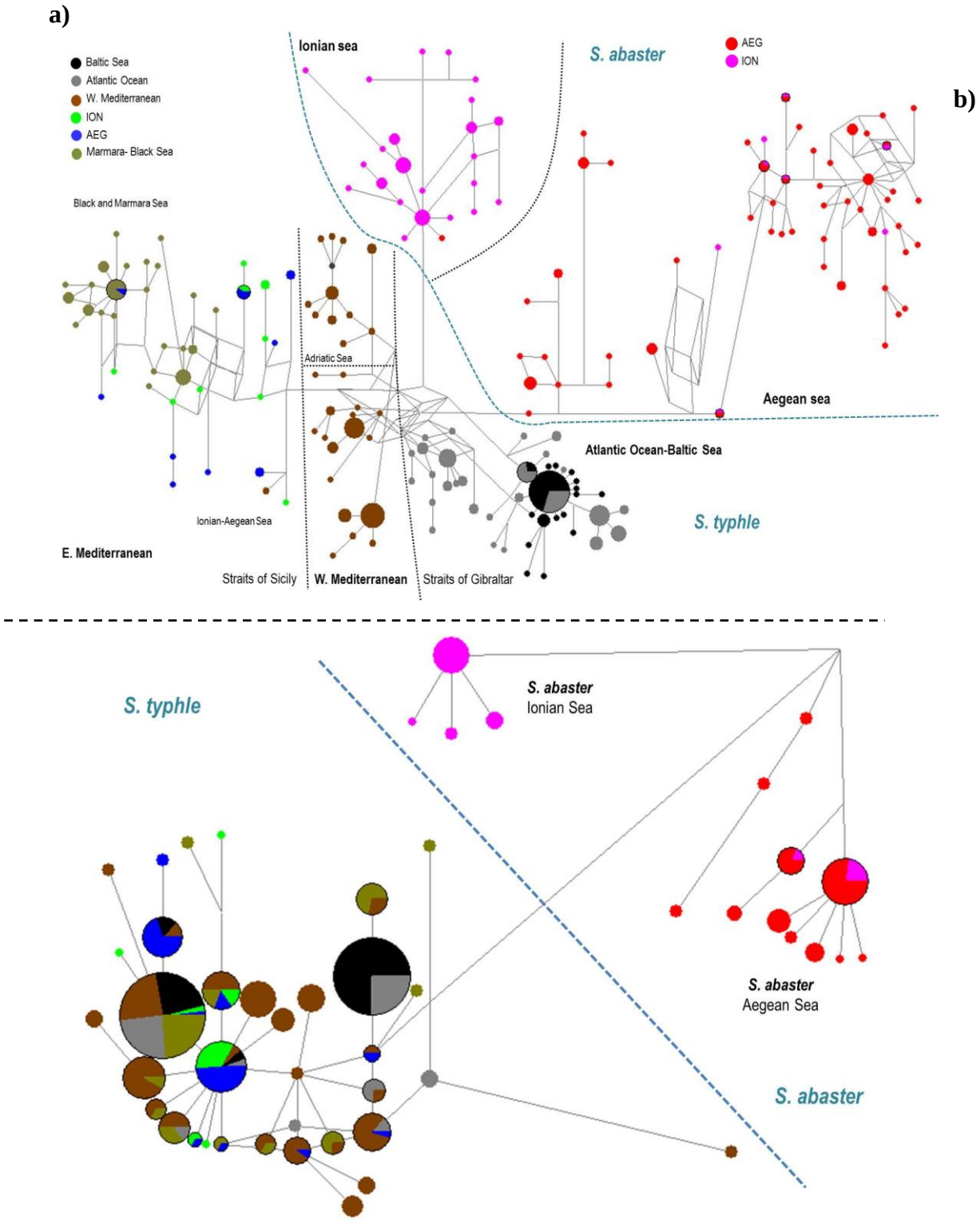


Figure 3.25. Median-joining network haplotypes of *S. abaster* and *S. typhle* European populations based on the analysis of a) mitochondrial Control Region and b) nuclear Locus A1. Circles are proportional to haplotype frequencies.

Εικόνα 3.25. Δίκτυο median-joining των σχέσεων των απλοτύπων των πληθυσμών των ειδών *S. abaster* και *S. typhle* στον ευρωπαϊκό χώρο, σύμφωνα με τα αποτελέσματα της ανάλυσης a) της περιοχής ελέγχου του μιτοχονδριακού DNA και b) του πυρηνικού τόπου A1. Το μέγεθος των κύκλων είναι ανάλογο της συχνότητας του κάθε απλοτύπου.

3.4. DISCUSSION

A species genetic structure is defined as the spatial and temporal distribution of genotypes (Hewitt 1999). By definition it is a complex equilibrium between processes favoring genetic homogeneity/ panmixia (gene flow) and genetic differentiation/ isolation (natural selection, drift and mutations) (Wright 1943; Slatkin 1985, 1987; Grant 1998; Bohonak 1999). These processes may stem from contemporary drives (environmental parameters or/and species-specific life history/ecological traits) or/ and past demographic events (climatic shifts and natural disasters leading to vicariant events, bottlenecks and founder effects etc.) (Avise et al. 1987; Avise, 2000; Hewitt 2000).

The most known contemporary drives are i. resource distribution and predation that leads to accumulation of large numbers of individuals in high quality territories (Slatkin 1987) and ii. dispersal ability. The general rule dictates that species with high dispersal ability exhibit genetic homogeneity and vice versa (Storz 1999; Ross 2001). However, exceptions to this rule started to gain ground (Hewitt 2000, Pelc et al. 2009). At the same time, the Ice Ages (especially the Pleistocene period) is probably the palaeoclimatic feature with the most defining impact on the genetic structure of numerous temperate species (e.g. Taberlet et al. 1998; Hewitt 2000; Provan and Bennett 2008; Wilson and Veraguth 2010; Alexandri et al. 2012, Zigouris et al. 2013) and is postulated to have shaped geographical patterns of genetic diversity. During the repeated cycles of glaciated and unglaciated activities Northern regions were mostly affected and many species inhabiting these areas went extinct or sought refugia in southern areas (Lomolino et al. 2006). These southern areas were the onset for a northward range expansion into old and new habitats mostly after last glacial maximum (LGM) (Hewitt 2000, Bernatchez and Wilson 1998; Hofreiter et al. 2004).

For marine ecosystems, the Pleistocene period is characterized by changes in the sea levels, which led to the drying of some estuaries and coastal areas (Durand et al. 2005). This change or even loss of near-shore reproductive habitats affected mostly intertidal, estuarine and nearshore- coastal species, while pelagic species are thought to be more resilient to glacial-induced habitat changes (Hellberg et al. 2001; Altman and Taylor 2003; Hickerson and Cunningham 2005).

Syngnathids, as near-shore animals, were affected by the repeated glacial interglacial cycles across their range. When the sea levels fell, they sought shelter in protected areas and postglacially managed to recolonize old and new habitats (mostly through repeated founders event), when the sea levels rose again (Chenoweth et al. 2002; Lourie and Vincent 2004; Mobley et al. 2011; Wilson 2006; Wilson and Veraguth 2010). As already stated, the two pipefishes (*S. abaster* and *S. typhle*) form distinct clades which reflect geographical affinities (Wilson and Veraguth 2010; Alaya et al. 2011; Sanna et al. 2013a).

The present study has given us valuable insights on the genetic structure and evolutionary patterns of *S. abaster* and *S. typhle* species both on a broader (European-Mediterranean coastline) and a more local (Greek coastline) scale. At a broader scale the

extended sampling of Greek seas reinforced the hypothesis that both *S. abaster* and *S. typhle* form well-defined clusters across their range (Wilson and Veraguth 2010; Alaya et al. 2011; Sanna et al. 2013a). On the contrary, along the Greek coastline the results of the population genetics analysis revealed a contrasting patterns of genetic structure between the two species i.e. strong population structure for *S. abaster* and shallower for *S. typhle*. These outcomes are discussed in the following pages.

3.4.1. Insight into the phylogeography of *S. abaster* and *S. typhle*.

At European level the work of Wilson and Veraguth (2010) has revealed a high degree of phylogeographic structure of *S. typhle* across its range, forming four clades which corresponded to major biogeographical zones: i) Baltic Sea- Atlantic Ocean, ii) W. Mediterranean, iii) Adriatic Sea iv) Marmara and Black Seas. mtDNA analysis in the present study showed that the Greek haplotypes belonged to distinct clades from the W. Mediterranean, Adriatic Marmara and Black Seas, forming thus an additional E. Mediterranean group (Figure 3a).

The distinct clades between the Greek and the Adriatic haplotypes did not come as a surprise. Several studies demonstrate i) that Adriatic Sea is a well-defined biogeographical zone within the Mediterranean (Bianchi 2007, 2012; Patarnello et al. 2007) and ii) the presence of a genetic break among the Adriatic and the Ionian Sea (e.g. Borsa et al. 1997; Tinti et al. 2002; Steffani and Thorley 2003, Gysels et al. 2004; Zardoya et al. 2004; Triantafyllidis et al. 2005; Teixeira et al. 2011; Sanna et al. 2013b). This pattern of genetic discontinuity is attributed to the vicariant event of Pleistocene (Bianchi 2007; 2012) and to a more contemporary hydrographical and circulation patterns i.e. temperature, salinity, tidal activities and gyres (Poulain 2001; Zardoya et al. 2004; Rolland et al. 2007; Patarnello et al. 2007).

Furthermore, many so far published studies indicate a reduction in the gene flow of Eastern and Western Mediterranean Sea populations for many marine species, even for those with high dispersal ability and large population size (e.g. Borsa et al. 1997; Bahri-Sfar et al. 2000; Stefanni and Thorley 2003; Suzuki et al. 2004; Sanna et al. 2013b). This pattern of differentiation has been attributed to the complex history of the Mediterranean Sea that was severely affected by the last glacial interglacial periods. The vast fluctuation of the sea levels altered the geomorphology of the coastline and combined with hydrological features (thermal and salinity structure, as well as circulation regimes, Millot 1999; Carlsson et al. 2004) ultimately led to the genetic clades of the eastern and western basins (e.g. Bahri-Sfar et al., 2000; Magoulas et al. 2006; Bianchi 2007). The Siculo-Tunisian Strait is one of the major genetic breaks separating western and eastern Mediterranean Sea (e.g. Borsa et al. 1997; Carlsson et al. 2004; Bianchi 2007; Sanna et al. 2013b). This genetic break seems to be also responsible for the separation of the Mediterranean populations of *S. typhle* and the formation of the eastern clade in which the Greek population belonged to.

Greek haplotypes were clearly separated from the W. Mediterranean and the Adriatic, with the exception of haplotype 61 (Figure 3a). This haplotype was observed by Wilson and Veraguth (2010), too. Since Greek samples were lacking in that study, they suggested that it was directly linked to the Marmara Sea population. However, the addition of the Greek haplotypes showed its affinity with the E. Mediterranean. This unique haplotype may indicate a more recent, though limited, gene exchange between E. Mediterranean and Adriatic Sea populations. A more far-fetched scenario is that this

haplotype may represent an early offshoot Adriatic lineage that introgressed an E. Mediterranean type mitochondrial genome before or during the beginning of the glacial-interglacials cycles. This lineage may have possibly persisted in the E Mediterranean refugium, and then recolonized the basin. It would be interesting to analyze more individuals along the Adriatic, Ionian and Aegean coastlines, in order to clarify whether this is a random event or not.

This outcome sheds more light in the species European- PontoCaspian tree, providing additional data on the direction of the species post glacial recolonization pattern. In particular, due to the many private alleles present in the Black Sea Wilson and Veraguth (2010) pondered if "*populations of S. typhle species had persisted in the Black Sea during its separation from the Sea of Marmara and Mediterranean or not*". Despite the addition of the Greek haplotypes most of these private alleles still exist. Therefore, it seems that a population could have indeed persisted in the Black Sea during the Pleistocene period.

At the same time, the presence of three Greek haplotypes (Hap 75, 96, 106) within the Marmara- Black sea complex indicate that: i) there could be a present restricted gene flow between the populations of the E. Mediterranean and the Marmama-Black Seas and/or ii) besides the population that might have persisted during the Pleistocene in the Black Sea, the basin could have also been recolonized from an eastern Mediterranean refugium.

In order to test these hypothesis a larger sample size of the Greek population, fossil data from the Black Sea and recolonization times of E. Mediterranean and Black Sea are necessary. However, none of these are available. Therefore, all scenarios are considered possible as they are also supported by similar patterns in other marine species i.e. *Engraulis encrasicolus* persisted in the Black Sea during glacial- interglacial events (Magoulas et al. 1996), *Platichthys flesus* recolonized Black Sea from an eastern Mediterranean refugium (Borsa et al. 1997), gene flow occurred between E. Mediterranean and Black Sea populations of *Cerastoderma glaucum* (Nikula and Vainola 2003).

At the same time the western and southern Mediterranean populations of *S. abaster* form well-defined clusters (Alaya et al. 2011; Sanna et al. 2013a). As already discussed, the available sequences of the W. Mediterranean populations could not be incorporated and compared to the Greek haplotypes in order to have a deeper insight into the species phylogeography. However, the high degree of phylogeographic structuring detected in southern and western. Mediterranean Sea populations (Alaya et al. 2011; Sanna et al. 2013a) and the remarkable regional structure in the Greek haplotypes revealed in the present study reinforce the indication that the species exhibits isolation by distance pattern at a broader scale. Whether the Ionian and/ or Aegean Sea populations group with any of the rest of the Mediterranean clades (Sanna et al. 2013a) or forms a cluster of their own needs to be further examined.

3.4.2. Contrasting patterns of genetic structure for *S. abaster* and *S. typhle*

At a more narrow scale (along the Greek coastline), both mtDNA and nDNA analyses revealed different population structure among the sympatric *S. abaster* and S. *typhle* (Figure 2, Table II). In most cases, a striking lack of haplotype sharing between Ionian and Aegean Sea individuals of *S. abaster* was observed as opposed to a shallower (even non-existing) population structure for *S. typhle* (Figure 2, Table II). The different levels of population structure between the two species indicate an incongruent post glacial recolonization pattern and subsequent evolution history.

In particular, both species, as littoral organisms, were most probably affected by the Pleistocene period. The successive glacial- interglacial circles that led to a retreat in the sea water level could have forced both species to abandon the Greek coastline and retreat in protected refugia. From that point onward the two species seem to have followed different paths. In particular, it is more likely that the individuals of *S. abaster* that postglacially recolonized the Ionian and Aegean Seas originated from at least two spatial separated refugia i.e. one for the Aegean and one for the Ionian Sea populations. On the other hand, the Greek population of *S. typhle* most probably originated from a common refugium located somewhere in the Eastern Mediterranean.

The possibility of two glacial refugia for the individuals of *S. abaster* is supported by the results of the Network (Figure 2a-b) and AMOVA (Table II) analyses but also by the information provided by the nucleotide (π) and haplotype (h) diversity indices (Table I). AMOVA and network analysis based on both mtDNA and nDNA markers revealed in most cases a lack of haplotype sharing among the individuals from the Ionian and Aegean Seas which led to a significant subdivision between the two seas populations and more regional groupings. Also, along the Greek coastline the observed high values of both indices based of both markers are within the range of those reported for other Mediterranean populations of *S. abaster* (Alaya et al. 2011; Sanna et al. 2013a) as well as other marine teleost species (Grant and Bowen 1998, Karaiskou et al. 2004). Such pattern is characteristic of i) secondary contact of previously isolated population or ii) large and stable populations through a significant time frame (Grant and Bowen 1998). Given that stable populations are typical of open sea or oceanic species and not of near shore -such as syngnathids-(Grant and Bowen 1998), the scenario of secondary contact is probably more suitable for *S. abaster.*

The difference in the genetic background of Ionian and Aegean Sea populations of *S. abaster* could have been sustained and enhanced by the limited dispersal ability of the species benthic juvenile and adult specimens (Silva et al. 2006). However, the existence of few shared haplotypes between the populations of the two Seas indicates that a limited gene flow was sustained even at a comparatively recent timescale, despite the geographical barriers, hydrological features and reduced dispersal ability. This restricted gene flow could be most probably attributed to passive transportation (Wilson 2006; Sanna et al.

2013a) but it seems that it was not sufficient to overcome the level of differentiation in the populations of the two Seas (Slatkin 1987; Pineda et al. 2007; Schunter et al. 2011).

The possibility of a common glacial refugium for the Greek population of *S. typhle* is supported by the results of Network (Figure 2c-d) and AMOVA (Table II) analyses based on both mtDNA and nDNA markers. mtDNA analysis revealed a significant level of differentiation among the studied populations (*Fst* = 0.203; P < 0.05). Despite the reduced haplotype sharing between the individuals of the Ionian and Aegean Seas genetic structure could not be attributed to a significant distinction between the individuals of the two Seas or to any other known geographical barrier. On the other hand, the results of nDNA analysis were incongruent with the genetic structuring revealed by the mitochondrial marker. According to the nuclear marker most haplotypes were shared between individuals from the studied localities, indicating high levels of connectivity and a gene flow stronger than any known geographical barrier that led to panmixia (*Fst* = 0.045; P > 0.05) (Table II).

A possible explanation for the common genetic background could be the existence of a common refugium i.e. a common gene pool, located somewhere in the Eastern Mediterranean- since Greek haplotypes of *S. typhle* form a clade of their one which is distinct from the Adriatic, W. Mediterranean and Marmara-Black Seas clades (Figure 3a). When the recolonization process was completed, the common genetic background could have been sustained due to the benthopelagic behavior of *S. typhle* specimens. In particular, despite the presence of the bony armor along the species body (Dawson 1986), the benthopelagic behavior of the species individuals- and hence the increased possibility for passive transportation- could have sustained a contemporary gene flow (e.g. Slatkin 1987; Pineda et al. 2007; Schunter et al. 2011). The resulting levels of gene flow seem to have overcome geographical barriers and led to gene exchange even among individuals from distant localities, such as the ones from Drepano (Ionian Sea) and Vassova (Aegean Sea).

The incongruent patterns of genetic structure along the European and the Greek coastline as revealed by both markers could be a result of their different evolutionary rate. Given that mitochondrial DNA evolves faster than nuclear (Brown et al. 1979, Caccone et al. 1999) the contradicting genetic structure could indicate a contemporary differentiation on the mtDNA that has not been depicted in the nuclear genome yet. A second hypothesis could be based on the different heritage line of both markers i.e. mtDNA is inherited through the maternal line while nDNA through both maternal and paternal (Meyer 1993). Therefore, the observed difference could imply that females have larger dispersal ability than males.

In conclusion the results of the present study indicate that the same vicariant event, glacial-interglacial cycles, and the different degree of dispersal ability are most probably responsible for the incongruent genetic structuring of *S. abaster* and *S. typhle* along the Greek coastline i.e. distinct Ionian and Aegean clades for *S. abaster* in contrast to the

shallower genetic structure uncorrelated to geographical barriers for *S. typhle*. A similar pattern has been observed in many congeneric and sympatrically occurring species worldwide (e.g. Dawson et al. 2002; Cabral et al. 2*000;* Bargelloni et al. 2005, 2008; Hickerson and Cunnigham 2005*;* Mokhtar et al. 2011; Felix Hackrad et al. 2013) underlining the importance of comparative phylogeographic studies in disentangling contemporary from past demographic effects (e.g. Dawson et al. 2002; Kelly and Palumbi 2010).

Chapter 4. Morphometric analysis of *Syngnathus abaster* and *Syngnathus typhle* species along the coastline of Greece

4.1. INTRODUCTION

In the last decades there is a growing interest in the evolutionary origins and pattern of diversification among syngnathids. The main tools to address this growing concern are the molecular and morphological techniques (e.g. Dawson 1986; Keivany and Nelson 2006; Mobley et al. 2011; Wilson and Orr 2011). Morphological analyses are mostly based on meristic variables (Dawson 1986; Hablutzel 2009; Alaya et al. 2011), specific length measurements (Dawson 1986; Lourie et al. 1999; Cakic et al. 2002; Thangaraj and Lipton 2011; Anderson 2012; Mwale et al. 2013) and some landmark-based morphometric characters (Leysen et al. 2011).

The present morphological taxonomy of European syngnathids is based on Dawson's Key, which uses meristic counts and general body features- particularly from the head- for identification (Dawson 1986). Two of the most well studied European species are *Syngnathus typhle* and *Syngnathus abaster* (Dawson 1986; Cakic et al. 2002; Ben-Amor 2007; Gürkan 2008; Alaya et al. 2011; Gürkan and Taskavac 2012).

Movčan (1988, according to Cakic et al. 2002) and Cakic et al. (2002) were the first who- based on morphometric and length measurements as well as meristic characters- proved the differences among marine and freshwater populations of syngnathids from the Black and Azov Seas and Danube River. The populations were mainly separated by length measurements, while meristic characters were not so important. Both studies suggested that the differences could be a phenotypic response to the habitats in which the populations live (Movčan 1988, Cakis et al. 2002).

Alaya et al. (2011) and Sanna et al. (2013a) highlighted the impact of environmental changes on phenotypic studies of *S. abaster*. Based on nine meristic characters Alaya et al. (2011) showed phenotypic divergence between a Tunisian and a French lagoon population but also among two subpopulations of the French lagoon. The segregation of French and Tunisian populations was also supported by molecular data. Sanna et al (2013) showed that meristic characters can discriminate W. Mediterranean populations of *S. abaster* species. This discrimination was also supported by molecular data.

Hablutzel (2009) was the first to compare the morphometry of the European pipefishes in order to explore the possibility of hybridization in the *Syngnathus* genus. In a comparative study between *Syngnathus typhle, Syngnathus taenionotus* and *Syngnathus rostellatus* species he indicated that morphometric analysis is a powerful tool in the discrimination of the three species.

Besides phylogenetic studies morphometric tools have also been used to prove sexual size dimorphism between male and female syngnathids. Gürkan and Taskavak (2012) showed that in *S. typhle* species head and body lengths are larger in females than males.

Compared to the progress that has been made on phylogenetic studies on a molecular level (Hablutzel 2009; Wilson and Veraguth 2010; Woodall et al. 2011; Alaya et al. 2011; Sanna et al. 2013a) it is obvious that morphometric analysis is in early stages. Even though, *S. abaster* (to a larger extent) and *S. typhle* (in a smaller) are well-known for their phenotypic variability, data at a macro- and microgeographical scale are rare and scare and limited to the above mentioned studies. However, morphometric techniques are widely used in ichthyology in order to detect patterns of phenotypic relationships between taxa (Strauss and Bookstein 1982; Cadrin 2000), to assess possible and effective hybridization events (Humphries 1984; Neff and Smith 1978), to differentiate populations and fish stocks (Reist 1985; Cadrin and Friedland 1999; Kassam et al. 2004; Ibanez et al. 2007; Gomez and Monteiro 2008) etc.

Moreover, in the era of truss protocol and geometric morphometric techniques, morphological analyses of the two species are mostly based on Dawson's key (1986). However, the meristic variables and the length measurements vary between the existing studies and comparisons among them are difficult. Also, since these measurements are highly correlated with size, much effort must be dedicated to size correction. Finally, linear distances cannot depict the complex shape of an organism leading to insufficient results (Adams 2004).

As already mentioned *S. abaster* and *S. typhle* species occur sympatrically along the Greek coastline. Existing studies across both species distributional range demonstrated that they occupy different ecological niches. In particular, *S. typhle* is a benthopelagic species feeding mostly on mobile and large pelagic preys (from Copepods to small size Gobiidae) (Malavasi et al. 2007, Oliveira et al. 2007). On the other side, *S. abaster* is a benthic species with a preference on little prey hidden in the vegetation (Malavasi et al. 2007; Franzoi et al. 2004). Different ecological niches of syngnathids have already been correlated with distinct morphological patterns (Kendrick and Hyndes 2005; Oliveira et al. 2007; Roos et al. 2009; Leysen et al. 2011; Van Wassenbergh et al. 2011)

Taking this factor into consideration, the first aim of the present chapter was to explore whether *S. abaster* and *S. typhle* are morphologically distinct or exhibit intermediate morphotypes. Given the above mentioned differences in the ecology of the two species they were expected to form two non-overlapping clades with no intermediate morphotypes.

The second goal of the present chapter was to check the morphological pattern of the two species along the Greek coastline. In fish species morphological variation is affected by trophic and environmental conditions (Corti et al. 1996; Clabaut et al. 2007). As already mentioned Ionian and Aegean Sea constitute different biogeographical zones, formed during the Pleistocene (Bianchi 2007) and are susceptible to different environmental factors (Coll et al. 2010). Therefore, distinct morphological patterns between the populations of both species in the Ionian and the Aegean Sea were expected to be found.

4.2. MATERIALS AND METHODS

4.2.1. Sampling stations

Specimens of *Syngnathus abaster* and *Syngnathus typhle* were collected along the mainland sublittoral zone of Greece and the Venice Lagoon (Figure 4.1, Table 4.1), using a hand net (mesh size of 2–4 mm). Samples were stored at 4% neutralized formalin solution. The localities depicted the range of the species distribution along the major biogeographical regions/ basins in the mainland coastline of Greece. In the laboratory, each specimen was identified to species level based on the key for European syngnathids (Dawson 1986).

For the morphological comparison of *S. abaster* and *S. typhle* species and the phenotypic variation of their populations, specimens from all sampling sites were analyzed. The sexual size dimorphism analysis was conducted in samples from Drepano and Neochori populations (Figure 4.1). Sex was determined macroscopically (existence of brood pouch) and -when needed- microscopically.

Figure 4.1. Map of Greece showing the sampling stations for the two studied species in the present study (● *S. typhle* Ionian Sea, ● *S. typhle* Aegean Sea, ● *S. abaster* Ionian Sea, ● *S. abaster* Aegean Sea). For number of samples see Table 4.2.

Εικόνα 4.1. Περιοχές δειγματοληψίας στην Ελλάδα για τα δύο μελετώμενα είδη κατά τη παρούσα μελέτη (● *S. typhle* Ιονίου Πελάγους, ● *S. typhle* Αιγαίου Πελάγους, ● *S. abaster* Ιονίου Πελάγους, ● *S. abaster* Αιγαίου Πελάγους). Ο αριθμός των συλλεχθέντων ατόμων παρουσιάζεται στον Πίνακα 4.2.

Table 4.1. Short description of the sampling stations along the sublittoral zone of Greece, from which samples of *S. abaster* and *S. typhle* species were collected during the present study
Πίνακας 4.1. Σύντομη περιγραφή των σταθμών δειγματοληψίας (κατά μήκος της υποπαραλιακής ζώνης της ηπειρωτικής ακτογραμμής της Ελλάδας) από τους οποίους συλλέχτηκαν τα άτομα των ειδών *S. abaster* και *S. typhle* της παρούσας μελέτης.

Station	**Ecosystem type**	**Exposure to the open sea**	**Sea grass coverage**
1.Drepano	Open Sea	Exposed	Patchy sea grass and bare sand
2.Neochori	Open Sea	Protected	Full coverage
3.Mitikas	Open sea	Exposed	Full coverage
4.Tourlida	Open Sea	Exposed	Full coverage
5.Kalogria	Estuaries	Protected	Full coverage
6.Katakolo	Open sea	Exposed	Full coverage
7.Kotichi	Estuaries	Protected	Full coverage
8.Moustos	Estuaries	Protected	Full coverage
9.Livanata	Open sea	Exposed	Patchy sea grass and bare sand
10.Karavomilos	Open sea	Exposed	Full coverage
11.Korinnos	Open sea	Exposed	Full coverage
12.Pilaia	Open sea	Exposed	Patchy sea grass and bare sand
13.Vourvourou	Open sea	Exposed	Full coverage
14.Porto Koufo	Open sea	Exposed	Full coverage
15.Vassova	Lagoon	Protected	Patchy sea grass and bare sand
16.Porto Lagos	Estuaries	Protected	Patchy sea grass and bare sand
17.Drana	Estuaries	Protected	Patchy sea grass and bare sand

4.2.2. Selected method

Anatomical and morphometric measurements have been traditionally used to assess intra and interspecific differences (e.g. Casselman et al. 1981; Ihssen et al. 1981; Reist 1985). Landmark-based geometric morphometric is an accurate method in which data can be collected in the form coordinates along a biological structure (Bookstein 1991; Monteiro and Reis 1999; Chen et al. 2005). Unlike analytical approaches, the landmark-based techniques test and visualize differences in form in a reproducible and statistically accurate way. Therefore, it is very effective in capturing information on the shape of an organism and measuring its populations' variation (Cavalcanti et al. 1999; O'Higgins 2000). In combination with multivariate statistical procedures, they constitute a reliable tool for the representation of body changes (Loy et al. 1993; Rohlf and Marcus 1993; Rohlf et al. 1996; Recasens et al. 2006).

4.2.3. Landmark- based morphometric protocol

For morphometric analysis, digital images of fish, in a standard position pointing to the left, were analyzed. Fifteen landmarks were digitized by image analysis software (NIKON Digital Sight DS-L2). Landmarks refer to: (P1) anterior tip of upper jaw, (P2) joint of upper and lower jaw, (P3) end of the snout, (P4) upper end of the head, (P5) lower end of the head, (P6) origin of dorsal fin, (P7) anus, (P8) end of dorsal fin, (P9) insertion of 1st dorsal caudal fin ray, (P10) insertion of 1st ventral caudal fin ray, (P11) midpoint of the origin of caudal fin, (P12) end of the caudal fin, (P13) upper end of the orbit, (P14) lower end of the orbit, (P15) center of orbit (Figure 4.2).

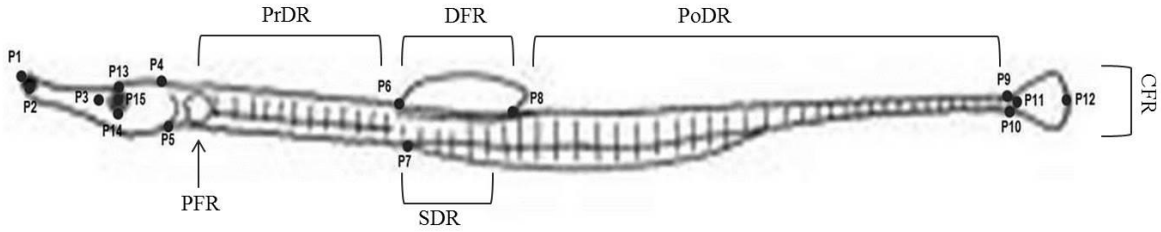


Figure 4.2. Landmarks and meristic characters used in the present study, shown on an outline drawing of *S. abaster* (*DFR*, number of predorsal rings; *SDR*, number of subdorsal rings; *PoDR*, number of postdorsal rings; *DFR*, number of dorsal fin rays; *CFR*, number of caudal fin rays; *PFR*, number of pectoral fin rays).

Εικόνα 4.2. Ορόσημα που χρησιμοποιήθηκαν για τη μορφομετρική ανάλυση στη παρούσα μελέτη απεικονισμένα σε περίγραμμα του είδους *S. abaster* (*DFR*, αριθμός δακτυλίων προ του ραχιαίου πτερυγίου; *SDR*, αριθμός δακτυλίων υπό του ραχιαίου πτερυγίου; *PoDR*, αριθμός δακτυλίων μετά το ραχιαίο πτερύγιο; *DFR*, αριθμός ακτίνων ραχιαίου πτερυγίου; *CFR*, αριθμός ακτίνων ουραίου πτερυγίου; *PFR*, αριθμός ακτίνων θωρακικού πτερυγίου).

4.2.4. Meristic protocol

Seven meristic characters were examined in each specimen, in order to describe the morphology of the two pipefish species (Cakic et al. 2002, Ben Amor et al. 2007, Gurkan 2008, Alaya et al. 2011): i.e. number of rays in dorsal fin, number of rays in each pectoral fin, number of rays in caudal fin, number of predorsal rings, number of subdorsal rings, number of postdorsal rings (Figure 4.2). The terminal ring was scored if a clear star-like structure of surface of the bone was visible.

4.2.5. Statistical analysis

Statistical analysis was the same for each data set (morphological comparison of *S. abaster* and *S. typhle* species, sexual dimorphism and population phenotypic variation). What differed was the number of the analyzed specimens. In the analysis of populations' phenotypic variation of *S. abaster* species various groupings were tested (1. Ionian, Aegean and Adriatic Sea 2. Aegean Sea, 3. Ionian Sea). In the analysis of *S. typhle* species only the Ionian, Aegean, Adriatic Sea grouping was tested due to i) the small number of Aegean Sea samples and i) the fact that all but one specimen from the Ionian Sea belonged to Drepano and Neochori populations and there would be no difference from the sexual dimorphism results.

4.2.5.1. Landmark based morphometric measurements

Body size (morphological pattern) was estimated as centroid size: the square root of the summed squared distances from each landmark to the configuration centroid, which is the average of all landmarks in a configuration (Bookstein 1991, Monteiro and Reis 1999). The raw coordinates of all specimens were aligned (i.e. translated, rotated, and scaled to match one another) using the Procrustes generalized orthogonal least-squares (GLS) superimposition method, which superimposes configurations by minimizing the sum of squared distances between corresponding landmarks (Rohlf and Slice 1990). The aligned landmark coordinates were used as shape variables in all subsequent computations.

A Principal Components Analysis (PCA) of the aligned coordinates was used to reduce the number of dimensions to the actual shape space dimensionality. The first $2p$-4 (p = number of landmarks) PC scores were used to ensure non-singularity of covariance matrices to be calculated in the discriminant analysis. Because PCA performs a rigid rotation of the space, no shape information is lost during this process. The PC scores were used as variables in the Linear Discriminant Analysis (LDA) (morphological comparison of *S. abaster* and *S. typhle* species) and the Canonical Variate Analysis (CVA) (sexual dimorphism and population phenotypic variation) in order to determine the reliability of specimen identification from shape scores. In both analyses, the discrimination

effectiveness was determined from the percentage of correct classifications in a leave-one-out cross validation for linear discriminant analysis (Venables and Ripley 2002).

MANOVA analysis (Sokal and Rohlf 1995) was used in order to investigate the effect of sex and different populations on sexual size dimorphism. PC scores were used as dependent variables and sex and populations as factors.

All morphometric and statistical analyses were performed with MorphoJ, R-project MASS package (Venables and Ripley 2002) and SPSS ver. 21.0.

4.2.5.2. Meristic characters

Spearman's rank correlation tests were conducted to exclude the possibility that size differences within a species could influence the results of subsequent analyses. The non-parametric test Mann-Whitney was applied on each meristic variable to test the occurrence of statistical significant differences between species, and sexes (Sokal and Rohlf 1995). The variance of meristic characters was not estimated in the population structure analysis, since i) sex was not available in all specimens, ii) there were cases of all-male and all-female populations. Statistical analyses were performed with SPSS ver. 21.0.

4.3. RESULTS

4.3.1. Morphological comparison of *Syngnathus abaster* and *Syngnathus typhle* species

A total of 483 specimens of *S. abaster* (n=333) and *S. typhle* (n=150) species were collected along the sublittoral zone of Greece (Figure 4.1, Table 4.2).

Table 4.2. Number of *S. abaster* and *S. typhle* specimens used in the morphometric analysis of the present study.
Πίνακας 4.2. Αριθμός συλληφθέντων ατόμων των ειδών *S. abaster* και *S. typhle* που χρησιμοποιήθηκαν στη μορφομετρική ανάλυση στη παρούσα μελέτη.

Sea	Sea Code	Geographical region	Sampling stations	Station Code	Number of individuals	
					S. abaster	*S. typhle*
Ionian Sea	ION	Port of Igoumenitsa	1.Drepano	DRE	99	49
		Amvrakikos Gulf	2.Neochori	AMV	51	55
		Mitikas	3.Mitikas	MIT	10	0
		Tourlida	4.Tourlida	TOU	6	0
			5.Kalogria	KAL	10	0
		Peloponesus	6.Katakolo	KAT	9	1
			7.Kotichi	KOT	10	0
Aegean Sea	AEG	Peloponesus	8.Moustos	MOU	10	0
		Evoikos Gulf	9.Livanata	LIV	3	4
			10.Karavomilos	KAR	21	0
		Thermaikos Gulf	11.Korinnos	KOR	23	3
			12.Pilaia	PIL	20	1
		Chalkidiki	13.Vourvourou	VOU	8	1
			14.Poto Koufo	PKO	5	0
		E. Macedonia	15.Vassova	VAS	16	10
		Thrace	16.Porto Lagos	PLA	10	0
			17.Drana	DRA	12	0
Adriatic Sea	ADR	Venice	18.Venice	ADR	10	26

4.3.1.1 Landmark based morphometric measurements

The PCA extracted 26 components. The first PC (PC I) accounted for 84.7% of total variance and was characterized by shape changes along the *x*-axis. More specifically, it showed an elongation of the snout, main body and caudal fin base. The second PC (PC II) accounted for 5.0 % of total variance and showed body changes along the y-axis. The rest of the PCs explained < 10.3% of total variance and described aspects of intraspecific shape variation. The PC scores were used as variables in the Linear Discriminant Analysis (LDA) to assess interspecific differences.

The structure of among-species shape variation was assessed by Linear Discriminant Analysis (LDA) of partial warps and uniform components. One Linear Function (LF 1) was extracted (Mahalanobis distance= 10.837, $p<0.0001$) for the two species. Species pair differences (in both Procrustes and Mahalanobis scale) were significant under permutation tests ($p < 0.001$). The cross-validation test (leave-one-out method) from Linear Discriminant Analysis correctly classified all cases (100%) proving that the two species were completely separated (Table 4.3).

Both analyses (PCA and LDA) showed a considerable difference among the species, defining two non-overlapping groups: i) *S. typhle* and ii) *S. abaster* species. The shape changes associated with the LF axes showed that *S. typhle* specimens had larger snout and main body, whereas their trunk was shorter compared to *S. abaster* (Figure 4.3).

Table 4.3. Classification results for the cross-validation procedure for the two studied *Syngnathus* species, using principal components of shape scores as variables in a linear discriminant analysis, in the present study.
Πίνακας 4.2. Επανατοποθέτηση των πληθυσμών των δύο υπό μελέτη ειδών του γένους *Syngnathus* σύμφωνα με τη γραμμική διαχωριστική ανάλυση, στην παρούσα μελέτη.

Species	**Predicted group membership**		
	S. abaster	*S. typhle*	*Total*
Count			
S. abaster	333	0	333
S. typhle	0	150	150
Percent %			
S. abaster	100.0	0.0	
S. tyhle	0.0	100.0	

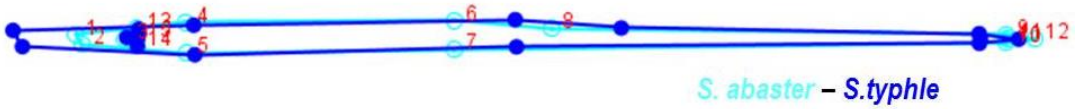


Figure 4.3. Observed shape changes associated with positive scores along the Principal Component Axes and the Discriminant Function Analysis. The wireframes depict icons for deviations (dark blue line) from the corresponding mean shape for *Syngnathus* species (light blue line) in the present study.
Figure 4.3. Παρατηρούμενη αλλαγή του σχήματος του σώματος με βάση τις θετικές τιμές του άξονα των Κυρίων Συνιστωσών και της Διαχωριστικής Ανάλυσης. Το πλέγμα απεικονίζει την απόκλιση (σκούρα μπλε γραμμή) από το σχήμα αναφοράς των δυο ειδών του γένους *Syngnathus* (ανοιχτή μπλε γραμμή) της παρούσας μελέτης.

4.3.1.2. Meristic characters

The number and range of the two species meristic characters are shown in Table 4.4. All characters were in accordance and within the range of each species as defined by Dawson (1986) and later studies (Movčan 1988; Cakic et al. 2002; Ben-Amor 2007; Gürkan 2008; Alaya et al. 2011; Gürkan and Taskavac 2012). Since the number of rays in the caudal fin did not vary among and between the species it was not considered for the rest of the analysis. The rest of the meristic characters were uncorrelated with SL within specimens of *S. abaster* and *S. typhe* species (Table 4.5). Kruskal- Wallis test showed that meristic characters differed statistically between the species (Dorsal Fin Rays: df=1, H=228.018, p<0.001; Pectoral Fin Ray_L: df=1, H=219.351, p<0.001; Pectoral Fin Rays_R: df=1, H=237.220, p<0.001; Predorsal rings: df=1, H=212.466, p<0.001; Underdorsal rings: df=1, H=205.143, p<0.001; Postdorsal rings: df=1, H=131.228, p<0.001).

Table 4.4. Values range and standard deviation of meristic characters of *S. abaster* and *S. typhle* individuals from the Ionian, Adriatic and Aegean Seas in the present study (N the value of meristic character).
Πίνακας 4.4. Τιμές, εύρος και τυπική απόκλιση των μεριστικών χαρακτήρων των ειδών *S. abaster* και *S. typhle* από το Ιόνιο και το Αιγαίο Πέλαγος καθώς και την Αδριατική Θάλασσα που χρησιμοποιήθηκαν στη παρούσα μελέτη (N η τιμή του μεριστικού χαρακτήρα).

Species	Meristic character	N	Range	Std. Deviation
S. abaster	Dorsal Fin Rays	28	23-32	2.175
	Pectoral Fin Ray_L	12	10-15	0.839
	Pectoral Fin Rays_R	12	10-15	0.785
	Caudal Fin Rays	10	10-10	0
	Predorsal Rings	15	11-18	1.103
	Underdorsal Rings	7	5-9	0.673
	Postdorsal Rings	28	21-34	2.148
S. typhle	Dorsal Fin Rays	33	28-37	1.732
	Pectoral Fin Ray_L	15	13-17	0.889
	Pectoral Fin Rays_R	15	13-17	0.888
	Caudal Fin Rays	10	10-10	0
	Predorsal Rings	18	15-21	1.310
	Underdorsal Rings	8	7-10	0.734
	Postdorsal Rings	24	20-30	1.851

Table 4.5. P-values for within *S. abaster* and *S. typhle* species correlations of meristic traits with Standard Length (SL) in the present study (Spearman's rank correlation test).
Πίνακας 4.5.Τιμές του ελέγχου συσχέτισης των μεριστικών χαρακτήρων με το Σταθερό Μήκος (SL) για τα είδη *S. abaster* και *S. typhle* της παρούσας μελέτης (Έλεγχος συσχέτισης κατά Spearman).

	Dorsal Fin Rays	**Pectoral Fin Rays_R**	**Pectoral Fin Rays_L**	**Predorsal Rings**	**Underdorsal Rings**	**Postdorsal Rings**
S. abaster	0.302	0.968	0.970	0.792	0.789	0.691
S. typhle	0.283	0.985	0.982	0.362	0.350	0.157

4.3.2. Sexual dimorphism of *Syngnathus abaster* and *Syngnathus typhle* species

A total of 216 specimens of *S. abaster* (n=129) and *S. typhle* (n=87) species were collected from the sampling stations of Drepano and Neochori. The number of females and males for each species in the two sampling stations is shown in Table 4.6.

Table 4.6. Number of male and female individuals of *S. abaster* and *S. typhle* species collected from Drepano and Neochori stations in the present study.
Πίνακας 4.6. Αριθμός αρσενικών και θηλυκών ατόμων των ειδών *S. abaster* και *S. typhle* που συλλέχτηκαν από τους σταθμούς του Δρεπάνου και του Νεοχωρίου στην παρούσα μελέτη.

Station	Sex	*S. abaster*	*S. typhle*
Drepano	Males	30	15
	Females	59	30
Neochori	Males	17	16
	Females	23	29

4.3.2.1. *Syngnathus abaster* species

4.3.2.1.1. Landmark based morphometric measurements

The PCA extracted 26 components. The first PC (PC 1) accounted for 59.0% of total variance and was characterized by shape changes along the *x*-axis. More specifically, it showed an elongation of the snout, trunk, dorsal and caudal fin base. The second PC (PC 2) accounted for 22.5 % of total variance and depicted the corresponding body changes along the y-axis. The rest of the PCs explain 18.5% of total variance and described aspects of intraspecific shape variation. The PC scores were used as variables in the Canonical Variate Analysis to assess sexual dimorphism.

The structure of among-sexes shape variation in the populations of Drepano and Neochori was assessed by the Canonical Variate Analysis (CVA) of partial warps and uniform components. Three Canonical Variates (CVs) were extracted. The first CV (CV 1) explained 67.6% of the total among-groups variance, whereas the second (CV 2) accounted for 25.6% (Figure 4.4). Values of Mahalanobis and Procrustes distances are shown in Table 3.7. The ordination of individual scores showed a considerable difference between the sexes and the population, defining four distinct groups: i) males of *S. abaster* from Neochori station, ii) females of *S. abaster* from Neochori station, iii) males of *S. abaster* from Drepano station and iv) females of *S. abaster* from Drepano station. The first two groups presented high positive scores and were separated from the last two along CV1.

Females presented higher positive scores than males along CV2. The shape changes associated with the CV axes showed that: i) individuals from Drepano had larger main body and snout compared to Neochori station, ii) females had more elongated main body and smaller trunk than males (Figure 4.5).

MANOVA analysis showed that the interaction of individual from different stations and sex was not significant (Wilk's lambda= 0.747, p>0.05). This means that males and females were different regardless of station, whereas individuals from different stations were different, regardless of sex. This was also evident in the CVA scatterplots as CV 1 was separating populations and the CV 2 was separating sexes.

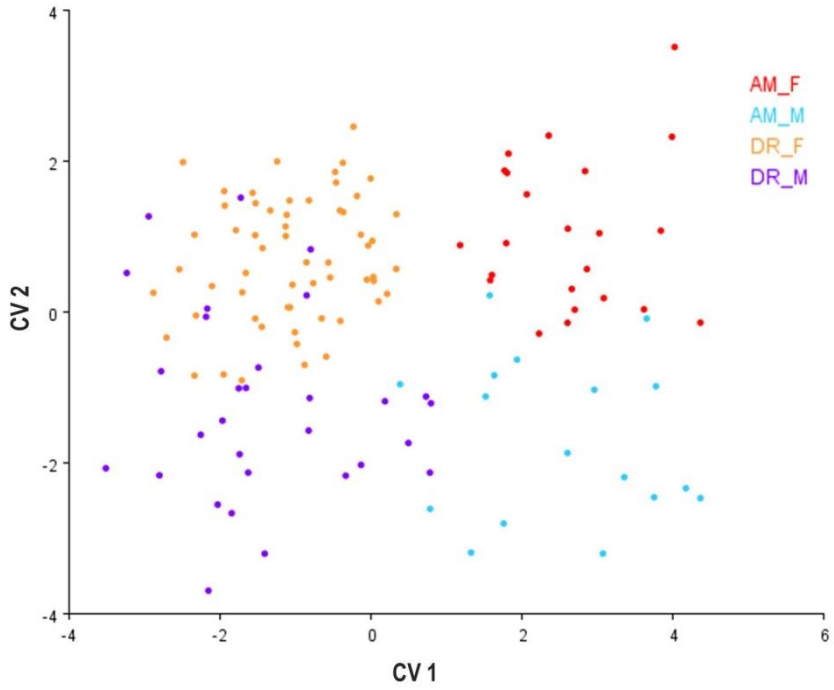


Figure 4.4. Scatter plot of the Canonical Variate scores for male and female individuals of *S. abaster* species from Neochori and Drepano stations of the preset study (AM_F: Females from Neochori station, AM_M: Males from Neochori station, DR_F: Females from Drepano station, DR_M: Males from Drepano station).
Εικόνα 4.4. Διάγραμμα διασποράς των τιμών της Ανάλυσης Κανονικών Συνιστωσών για τα θηλυκά και αρσενικά άτομα του είδους *S. abaster* από τους σταθμούς του Νεοχωρίου και του Δρεπάνου της παρούσας μελέτης (AM_F: Θηλυκά άτομα από τον σταθμό του Νεοχωρίου, AM_M: Αρσενικά άτομα από τον σταθμό του Νεοχωρίου, DR_F: Θηλυκά άτομα από τον σταθμό του Δρεπάνου, DR_M: Αρσενικά άτομα από τον σταθμό του Δρεπάνου).

Table 4.7. Values of (a) Mahalanobis and (b) Procrustes distances for male and female individuals of *S. abaster* species from Neochori and Drepano stations in the preset study (AM_F: Females from Neochori station, AM_M: Males from Neochori station, DR_F: Females from Drepano station, DR_M: Males from Drepano station).
Πίνακας 4.7. Τιμές της γεωμετρικής απόστασης κατά α) Mahalanobis και β) Procrustes στην Ανάλυσης Κανονικών Συνιστωσών για τα θηλυκά και αρσενικά άτομα του είδους *S. abaster* από τους σταθμούς του Νεοχωρίου και του Δρεπάνου της παρούσας μελέτης (AM_F: Θηλυκά άτομα από τον σταθμό του Νεοχωρίου, AM_M: Αρσενικά άτομα από τον σταθμό του Νεοχωρίου, DR_F: Θηλυκά άτομα από τον σταθμό του Δρεπάνου, DR_M: Αρσενικά άτομα από τον σταθμό του Δρεπάνου).

	a)			**b)**		
	AM_F	AM_M	DR_F	AM_F	AM_M	DR_F
AM_M	3.0721*			0.0166		
DR_F	3.814*	4.2943*		0.0239*	0.0381*	
DR_M	4.6236*	4.1822*	2.1947*	0.0117	0.0242*	0.0155

*Statistical significant differences, P <0.05; *Στατιστικά σημαντικές διαφορές, P <0.05.

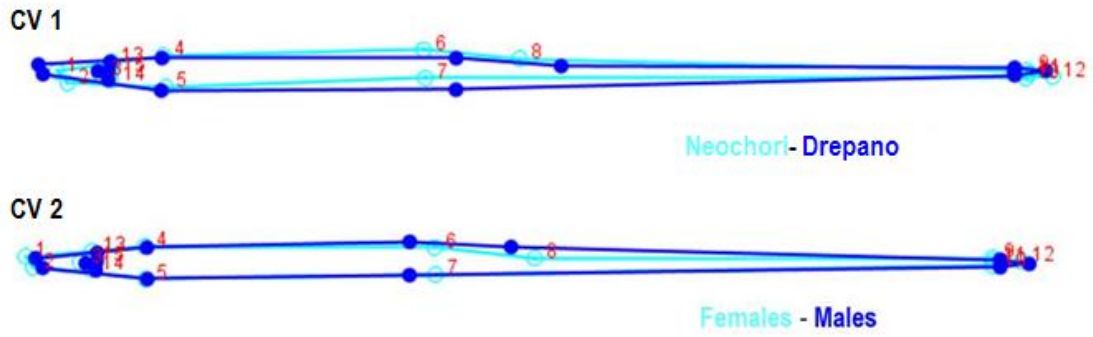


Figure 4.5. Observed shape changes associated with positive scores along the Canonical Variate Axes in the sexual dimorphism analysis of *S. abaster* species of the present study. The wireframes depict icons for deviations (dark blue line) from the corresponding mean shape of *S. abaster* species (light blue line).
Εικόνα 4.5. Παρατηρούμενη αλλαγή του σχήματος του σώματος με βάση τις θετικές τιμές του άξονα των Κανονικών Συνιστωσών για την ανάλυση φυλετικού διμορφισμού του είδους *S. abaster* της παρούσας μελέτης. Το πλέγμα απεικονίζει την απόκλιση (σκούρα μπλε γραμμή) από το σχήμα αναφοράς του είδους *S. abaster* (ανοιχτή μπλε γραμμή).

4.3.2.1.2. Meristic characters

The values and range of meristic characters for male and female specimens of *S. abaster* species from Drepano and Neochori stations are shown in Table 4.8. Since the number of rays in the caudal fin did not vary among and between the sexes they were not considered in the rest of the analysis.

MANOVA analysis indicated that the meristic character separating males and females of *S. abaster* species was the number of postdorsal rings (more in males than in females) (Table 4.8). The two populations were separated for almost every meristic character, but the number of Doral fin rays (Table 4.9). However, contrary to the morphometric measurements, the interaction of station and sex was significant (Table 4.9).

Table 4.8. Values, range and standard deviation of meristic characters for male and female specimens of *S. abaster* species from Drepano and Neochori stations in the present study (N, the value of each meristic character).
Πίνακας 4.8. Τιμές, εύρος και τυπική απόκλιση των μεριστικών χαρακτήρων των αρσενικών και θηλυκών ατόμων του είδους *S. abaster* από τους σταθμούς του Νεοχωρίου και του Δρεπάνου της παρούσας μελέτης (N, η τιμή του εκάστοτε μεριστικού χαρακτήρα).

Sex	Station	Meristic character	N	Range	Std. Deviation
Males	Drepano	Dorsal Fin Rays	27	23-32	1.815
		Pectoral Fin Rays _L	12	11-15	0.945
		Pectoral Fin Rays _R	12	11-13	0.634
		Caudal Fin Rays	10	10-10	0.000
		Predorsal Rings	15	13-18	1.154
		Underdorsal Rings	7	6-8	0.675
		Postdorsal Rings	27	25-31	1.386
	Neochori	Dorsal Fin Rays	28	23-30	1.759
		Pectoral Fin Rays _L	12	11-12	0.479
		Pectoral Fin Rays _R	12	11-13	0.809
		Caudal Fin Rays	10	10-10	0.000
		Predorsal Rings	15	13-17	0.862
		Underdorsal Rings	6	5-7	0.606
		Postdorsal Rings	28	25-31	1.663
Females	Drepano	Dorsal Fin Rays	27	23-32	1.802
		Pectoral Fin Rays _L	12	11-14	0.862
		Pectoral Fin Rays _R	12	10-14	0.925
		Caudal Fin Rays	10	10-10	0.000
		Predorsal Rings	15	13-18	0.841
		Underdorsal Rings	7	6-8	0.641
		Postdorsal Rings	27	25-32	1.618
	Neochori	Dorsal Fin Rays	27	24-29	1.153
		Pectoral Fin Rays _L	12	10-13	0.785
		Pectoral Fin Rays _R	12	10-12	0.561
		Caudal Fin Rays	10	10-10	0.000
		Predorsal Rings	15	13-16	0.665
		Underdorsal Rings	6	6-8	0.590
		Postdorsal Rings	27	25-32	1.792

Table 4.9. MANOVA analysis results of meristic characters for male and female specimens of *S. abaster* species from Drepano and Neochori stations in the present study (*, interaction)
Πίνακας 4.9. Αποτελέσματα της ανάλυσης AMOVA των μεριστικών χαρακτήρων των αρσενικών και θηλυκών ατόμων του είδους *S. abaster* από τους σταθμούς του Νεοχωρίου και του Δρεπάνου της παρούσας μελέτης (*, αλληλεπίδραση).

	Station		Sex		Sex* Station	
Meristic character	**F**	**P values**	**F**	**P values**	**F**	**P values**
Dorsal Fin Rays	3.506	< 0.05	0.031	> 0.05	0.575	> 0.05
Pectoral Fin Rays _L	4.871	< 0.05	0.520	> 0.05	1.725	> 0.05
Pectoral Fin Rays _R	5.028	< 0.05	0.146	> 0.05	0.004	> 0.05
Predorsal Rings	12.312	< 0.001	0.586	> 0.05	0.041	> 0.05
Underdorsal Rings	13.341	< 0.001	0.889	> 0.05	0.250	> 0.05
Postdorsal Rings	5.617	< 0.05	4.063	< 0.05	1.453	> 0.05

4.3.3.2. *Syngnathus typhle* species

4.3.2.2.1. Landmark based morphometric measurements

The PCA extracted 26 components. The first PC (PC I) accounted for 52.4% of total variance and was characterized by elongation of snout, base of dorsal fin, trunk and caudal fin along the *x*-axis. The second PC (PC II) accounted for 16.8 % of total variance and depicted the corresponding body changes along the y-axis. The rest of the PCs explained 30.8% of total variance and described aspects of intraspecific shape variation. The PC scores were used as variables in the Canonical Variate Analysis to assess sexual dimorphism.

The structure of among-sexes shape variation in the stations of Drepano and Neochori was assessed by Canonical Variate Analysis (CVA) of partial warps and uniform components. Three Canonical Variates (CVs) were extracted. The first CV (CV 1) explained 47.9% of the total among-groups variance, whereas the second (CV 2) accounted for 40.7% (Figure 4.6). Values of Mahalanobis and Procrustes distances are shown in Table 3.10. The ordination of individual scores showed a considerable difference both between the sexes and stations, defining four distinct groups: i) males of *S. typhle* from Neochori station, ii) females of *S. typhle* from Neochori station, iii) males of *S. typhle* from Drepano station and v) females of *S. typhle* from Drepano station. The first two groups presented high negative scores and were separated from the last two along CV1. Females presented negative scores, lower than males along CV2. The shape changes associated with the CV axes showed that: i) females had a more elongated main body and snout as opposed

to males who had a more elongated trunk, ii) individuals from Drepano had more elongated trunk compared to Neochori population (Figure 4.7) .

MANOVA analysis indicated that the interaction of different populations and sex was not significant (Wilk's lambda= 0.652, p>0.05). This means that males and females were different regardless of station whereas individuals from different stations were different, regardless of sex.

Table 4.10. Values of a) Mahalanobis and b) Procrustes distances for male and female individuals of *S. typhle* species from the Neochori and Drepano stations in the present study (AM_F: Females from Neochori station, AM_M: Males from Neochori station, DR_F: Females from Drepano station, DR_M: Males from Drepano station, *, level of significance >0.05).
Πίνακας 4.10. Τιμές της γεωμετρικής απόστασης κατά α) Mahalanobis και β) Procrustes στην Ανάλυσης Κανονικών Συνιστωσών για τα θηλυκά και αρσενικά άτομα του είδους *S. typhle* από τους σταθμούς του Νεοχωρίου και του Δρεπάνου της παρούσας μελέτης (AM_F: Θηλυκά άτομα από τον σταθμό του Νεοχωρίου, AM_M: Αρσενικά άτομα από τον σταθμό Νεοχωρίου, DR_F: Θηλυκά άτομα από τον σταθμό του Δρεπάνου, DR_M: Αρσενικά άτομα από τον σταθμό του Δρεπάνου).

	a)			**b)**		
	AM_F	AM_M	DR_F	AM_F	AM_M	DR_F
AM_M	3.027*			0.0235*		
DR_F	3.078*	3.022*		0.0162*	0.0344*	
DR_M	2.998*	3.057*	3.015*	0.0163	0.0121	0.0247*

*Statistical significant differences, P <0.05; *Στατιστικά σημαντικές διαφορές, P <0.05.

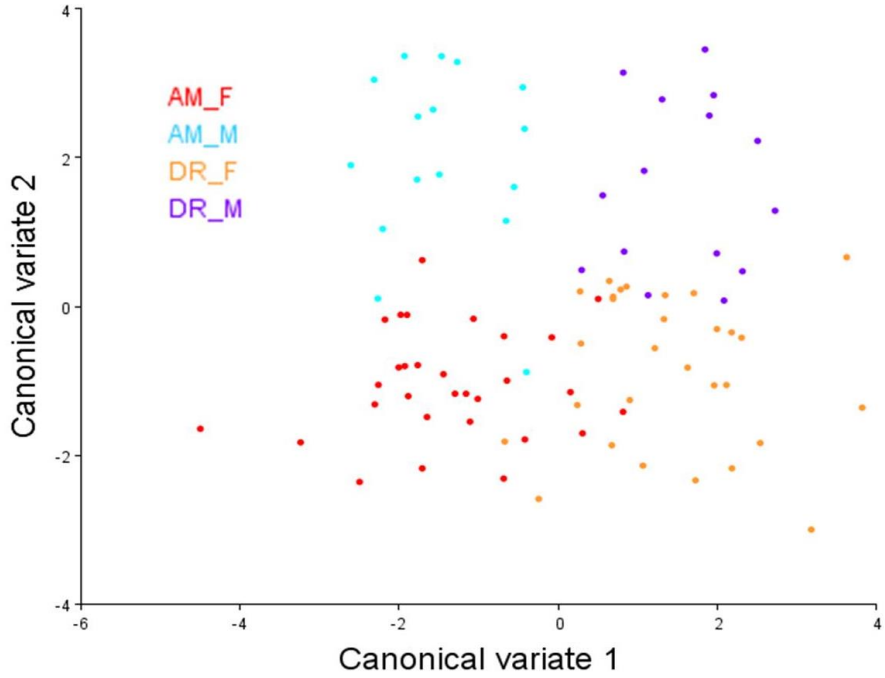


Figure 4.6. Scatter plot of the Canonical Variate scores for male and female individuals of *S. typhle* species from the Neochori and Drepano stations in the present study (AM_F: Females from Neochori population, AM_M: Males from Neochori station, DR_F: Females from Drepano station, DR_M: Males from Drepano station).

Εικόνα 4.6. Διάγραμμα διασποράς των τιμών της Ανάλυσης Κανονικών Συνιστωσών για τα θηλυκά και αρσενικά άτομα του είδους *S. typhle* από τους σταθμούς του Νεοχωρίου και του Δρεπάνου, της παρούσας μελέτης (AM_F: Θηλυκά άτομα από τον σταθμό του Νεοχωρίου, AM_M: Αρσενικά άτομα από τον σταθμό του Νεοχωρίου, DR_F: Θηλυκά άτομα από τον σταθμό του Δρεπάνου, DR_M: Αρσενικά άτομα από τον σταθμό του Δρεπάνου).

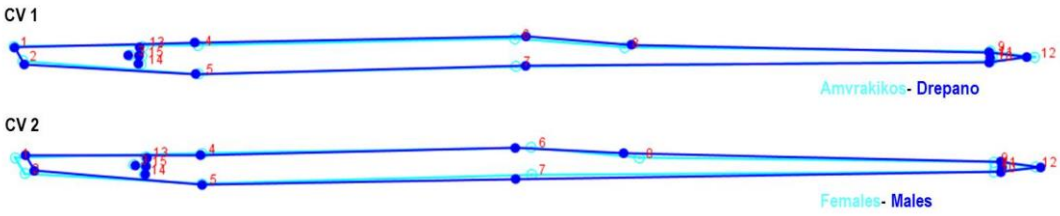


Figure 4.7. Observed shape changes associated with positive scores along the Canonical Variate Axes in the sexual dimorphism analysis of *S. typhle* species from the stations of Neochori and Drepano of the present study. The wireframes depict icons for deviations (dark blue line) from the corresponding mean shape of *S. typhle* species (light blue line).

Εικόνα 4.7. Παρατηρούμενη αλλαγή του σχήματος του σώματος με βάση τις θετικές τιμές του άξονα των Κανονικών Συνιστωσών για την ανάλυση φυλετικού διμορφισμού του είδους *S. typhle* από τους σταθμούς του Νεοχωρίου και του Δρεπάνου της παρούσας μελέτης. Το πλέγμα απεικονίζει την απόκλιση (σκούρα μπλε γραμμή) από το σχήμα αναφοράς του είδους *S. typhle* (ανοιχτή μπλε γραμμή).

4.3.2.2.2. Meristic characters

The values and range of meristic characters for male and female specimens of *S. typhle* species from Drepano and Neochori stations are shown in Table 3.11. Since the number of rays in the caudal fin did not vary among and between the sexes it was not considered for the rest of the analysis. MANOVA analysis showed that the meristic character separating males and females of *S. typhle* species was the number of predorsal rings (higher number in males than females) (Table 4.12). The individuals from the two stations were separated only by the number of predorsal rings, (Table 4.12). However, as in the case of meristic characters of *S. abaster* species, the interaction of individuals from different stations and sexes was significant (Table 4.12). This means that meristic characters of males and females varied along the stations.

Table 4.11. Values, range and standard deviation of meristic characters for male and female specimens of *S. typhle* species from Drepano and Neochori stations in the present study (N, value of each meristic character).
Πίνακας 4.11. Τιμές, εύρος και τυπική απόκλιση των μεριστικών χαρακτήρων των αρσενικών και θηλυκών ατόμων του είδους *S. typhle* από τους σταθμούς του Δρεπάνου και του Νεοχωρίου της παρούσας μελέτης (N, η τιμή του εκάστοτε μεριστικού χαρακτήρα).

Sex	Station	Meristic character	N	Range	Std. Deviation
Males	Drepano	Dorsal Fin Rays	32	30-34	1.261
		Pectoral Fin Rays _L	15	13-17	0.997
		Pectoral Fin Rays _R	15	13-17	1.027
		Caudal Fin Rays	10	10-10	0.000
		Predorsal	18	16-20	0.941
		Underdorsal	8	7-9	0.458
		Postdorsal	24	21-26	1.454
	Neochori	Dorsal Fin Rays	32	28-38	1.592
		Pectoral Fin Rays _L	15	14-17	0.877
		Pectoral Fin Rays _R	15	14-16	0.730
		Caudal Fin Rays	10	10-10	0.000
		Predorsal Rings	17	16-18	0.633
		Underdorsal Rings	8	7-9	0.616
		Postdorsal Rings	24	22-25	0.770
Females	Drepano	Dorsal Fin Rays	32	29-36	1.513
		Pectoral Fin Rays _L	15	14-17	0.745
		Pectoral Fin Rays _R	15	15-17	0.570
		Caudal Fin Rays	10	1010	0.000
		Predorsal Rings	17	15-20	1.200
		Underdorsal Rings	8	7-9	0.650
		Postdorsal Rings	23	21-26	1.315
	Neochori	Dorsal Fin Rays	33	28-35	1.554
		Pectoral_L Fin Rays	15	13-17	0.971
		Pectoral Fin Rays _R	15	13-17	1.100
		Caudal Fin Rays	10	10-10	0.000
		Predorsal Rings	17	15-19	0.937
		Underdorsal Rings	8	7-9	0.786
		Postdorsal Rings	24	22-25	0.941

Table 4.11. MANOVA analysis results of meristic characters for male and female specimens of *S. typhle* species from Drepano and Neochori stations in the present study.
Πίνακας 4.11. Αποτελέσματα της ανάλυσης AMOVA των μεριστικών χαρακτήρων των αρσενικών και θηλυκών ατόμων του είδους *S. typhe* από τους σταθμούς του Δρεπάνου και του Νεοχωρίου της παρούσας μελέτης.

	Station		**Sex**		**Station*sex**	
	F	**P-values**	**F**	**P-values.**	**F**	**P-values**.
Dorsal Fin rays	0.011	< 0.05	0.161	< 0.05	2.791	< 0.05
Pectoral Fin rays _L	1.196	< 0.05	0.453	< 0.05	1.196	< 0.05
Pectoral Fin rays_R	4.016	> 0.05	0.355	< 0.05	0.661	< 0.05
Predorsal Rings	3.945	> 0.05	4.336	> 0.05	2.921	< 0.05
Underdorsal Rings	0.148	< 0.05	2.739	< 0.05	0.003	< 0.05
Postdorsal Rings	3.553	< 0.05	3.341	< 0.05	.0203	< 0.05

4.3.3. Phenotypic variation of individuals of *Syngnathus abaster* and *Syngnathus typhle* species from different stations

4.3.3.1. *Syngnathus abaster* species

A total of 333 specimens of *S. abaster* were collected along the sublittoral zone of Greece (n=323) and the Venice Lagoon (n=10) (Figure 4.1, Table 4.2). The PCA extracted 26 components (PCs). The first PC (PC 1) accounted for 44.7% of total variance, the second PC (PC 2) for 24.5% and the third PC (PC 3) for 10.9%. These three PCs characterized shape changes of head, body and caudal fin mostly along the *x*-axis (PC1 and 3) and to a smaller extend along the y-axis (PC 2). The rest of the PCs explain 19% of total variance and described trivial aspects of intraspecific shape variation. The PC scores were used as variables in the Canonical Variate Analysis to assess population structure.

Table 4.13. P- values for a) Mahalanobis and b) Procrustes distances for the morphometric structure analysis of *S. abaster* species between a) Adriatic, Ionian and Aegean Sea, b) Ionian Sea stations and c) Aegean Sea stations in the present study (station codes after Table 4.2) (*Statistical significant differences, P <0.05).

Πίνακας 4.13. Τιμές της γεωμετρικής απόστασης κατά Mahalanobis και Procrustes κατά την ανάλυση της μορφομετρικής δομής του είδους *S. abaster* μεταξύ των σταθμών a) της Αδριατικής Θάλασσας, του Ιονίου και του Αιγαίου, b) του Ιονίου και c) του Αιγαίου Πελάγους, της παρούσας μελέτης (οι κωδικοί των σταθμών σύμφωνα με τον Πίνακα 4.2) (*,Στατιστικά σημαντικές διαφορές, P <0.05).

a)	**Mahalanobis**		**Procrustes**	
	ADR	AEG	ADR	AEG
AEG	3.0282 *		0.0148 *	
ION	3.6106 *	1.5948 *	0.0131 *	0.0063 *

b)

Mahalanobis distance	AMV	DRE	KAL	KAT	KOT	MI
DRE	3.7984*					
KAL	4.0848*	4.9343*				
KAT	5.8003*	4.2119*	6.5877*			
KOT	4.5292*	4.6013*	2.7118	6.7948*		
MIT	3.5521*	2.4898*	4.4222*	4.9387*	4.7236*	
TOU	5.8843*	3.5657*	6.2242*	5.0378*	6.4758*	3.9753*
Procrustes distance	AMV	DRE	KAL	KAT	KOT	MIT
DRE	0.0245*					
KAL	0.0281*	0.0194*				
KAT	0.0394*	0.0250*	0.0189			
KOT	0.0231*	0.0132	0.0107	0.0225*		
MIT	0.0180*	0.0130	0.0183*	0.0296*	0.0152	
TOU	0.0397*	0.0220*	0.0163	0.0195	0.0206*	0.0247*

Table 4.13. Continued
Πίνακας 4.13 Συνέχεια

c)

Mahalanobis distance	DRA	KAR	KOR	LIV	MOU	PIL	PKO	VAS	VIS
KAR	4.2756*								
KOR	4.5205*	2.9311*							
LIV	7.0923*	4.5838*	4.0277*						
MOU	7.507*	6.847*	8.0746*	9.0866*					
PIL	4.3856*	2.8549*	2.2222*	4.5967*	7.4605*				
PKO	5.4485*	3.2286*	3.9705*	5.6124*	6.3586*	3.8996*			
VAS	4.2034*	3.0254*	3.4409*	5.5408*	7.8712*	3.6207*	3.9362*		
VIS	3.0634*	4.2051*	4.7235*	6.975*	7.386*	4.5788*	4.683*	3.5512*	
VOU	5.981*	3.025*	4.4272*	5.3314*	5.6249*	4.3774*	3.4989*	4.7067*	5.9005*
Procrustes distance	DRA	KAR	KOR	LIV	MOU	PIL	PKO	VAS	VIS
KAR	0.0124*								
KOR	0.01	0.0094*							
LIV	0.015	0.0116	0.0117						
MOU	0.0361*	0.034*	0.0322*	0.0373					
PIL	0.0124*	0.0101*	0.0077	0.0167	0.0301*				
PKO	0.0157	0.0118	0.0117	0.0169	0.0253	0.0085			
VAS	0.0085	0.0103*	0.0089	0.015	0.0323*	0.0092	0.0119		
VIS	0.0094	0.0108	0.0105	0.0185	0.0307*	0.0074	0.0103	0.0077	
VOU	0.0165*	0.0092	0.0121	0.0129	0.0277*	0.013	0.0097	0.0119	0.0138

More specifically, the morphometric structure of *S. abaster* species was assessed by the Canonical Variate Analysis (CVA) of partial warps and uniform components. Seventeen Canonical Variates (CVs) were extracted. The first four CVs accounted for the 74.1% of total among groups variance (CV 1: 37.5%; CV 2: 15.8%; CV 3: 12.4%; CV 4: 8.5%). Despite the overlap of some stations, values of Mahalanobis and Procrustes distances indicated a strong morphological differentiation among the individuals from the stations in the Greek coastline and between the Ionian, Aegean and the Adriatic Sea specimens (Table 4.13a). Taking this into account and the ordination of individual scores, three distinct groups were revealed: i) Aegean Sea population, ii) Ionian Sea population and iii) Adriatic Sea (Venice Lagoon) populations CV 1 separated the Ionian population from the Adriatic and Aegean, while CV 2 Adriatic population from the population of Greece (Figure 3.8). The shape changes associated with the CV axes showed that: i) the population of the Ionian Sea had a more slender body and elongated snout compared to the Aegean Sea, ii) the population of Adriatic Sea had a more slender body and shorter snout compared to the Greek (Figure 4.9).

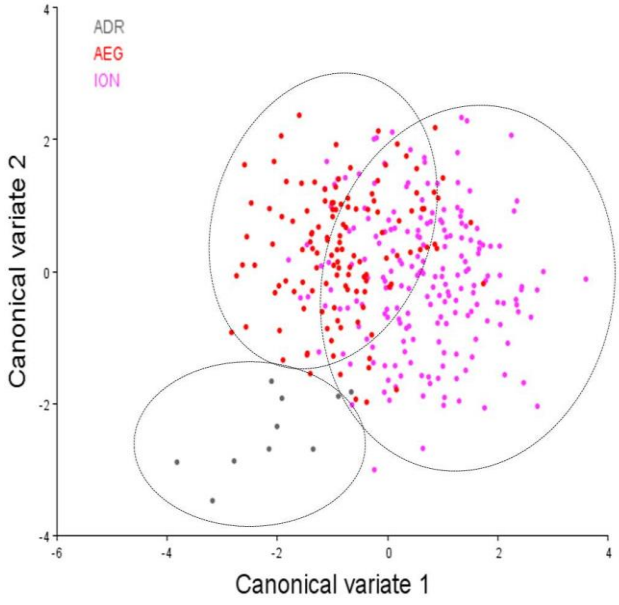


Figure 4.8. Scatter plot of the Canonical Variate scores of the morphometric structure analysis for Adriatic, Ionian and Aegean individuals of *S. abaster* species in the present study (stations codes after Table 4.2).
Εικόνα 4.8. Διάγραμμα διασποράς των τιμών της Ανάλυσης Κανονικών Συνιστωσών της ανάλυσης της μορφομετρικής δομής των πληθυσμών του είδους *S. abaster* από τους σταθμούς της Αδριατικής Θάλασσας, του Ιονίου και του Αιγαίου Πελάγους (οι κωδικοί των σταθμών σύμφωνα με τον Πίνακα 4.2).

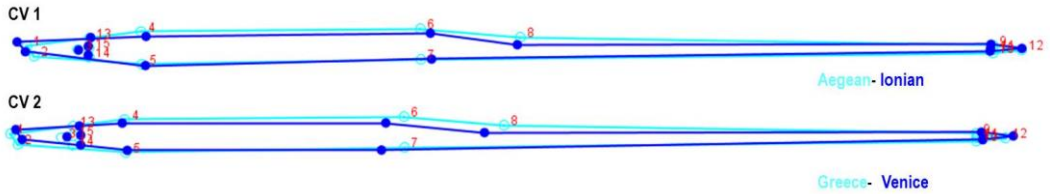


Figure 4.9. Observed shape changes associated with positive scores along the Canonical Variate Axes of the morphometric structure analysis for Adriatic, Ionian and Aegean individuals of *S .abaster*. The wireframes depict icons for deviations (dark blue line) from the corresponding mean shape for *S. abaster* species in the present study (light blue line).
Εικόνα 4.9. Παρατηρούμενη αλλαγή του σχήματος του σώματος με βάση τις θετικές τιμές του άξονα των Κανονικών Συνιστωσών της ανάλυσης της μορφομετρικής δομής του είδους *S. abaster* από τους σταθμούς της Αδριατικής Θάλασσας, του Ιονίου και του Αιγαίου Πελάγους. Το πλέγμα απεικονίζει την απόκλιση (σκούρα μπλε γραμμή) από το σχήμα αναφοράς του είδους *S. abaster* κατά την παρούσα μελέτη (ανοιχτή μπλε γραμμή).

For the population of the Ionian Sea six CVs were extracted from the Canonical Variate Analysis. The first three explained 87.9 % of among group total variance (CV 1: 53.7%; CV 2: 21.6%; CV 3: 12.6%). Mahalanobis and Procrustes distances (Table 4.13.b), as well as, the ordination of individual scores showed a considerable difference between populations defining three distinct groups: i) N. Ionian Sea- Katakolo group, ii) Kotichi-Kalogria group and iii) Neochori (Amvrakikos Gulf) (Figure 4.10). The first group had lower scores along CV1 and was separated from the other two. Kotichi-Kalogria group had higher scores along the CV 2 and was separated from the other two. The shape changes associated with the CV axes showed that: i) individuals from Kotiki- Kalogria and Neochori group had a more elongated body and a thicker trunk than N. Ionian Sea-Katakolo group, ii) individuals from Kalogria- Kotichi group had a larger head, a more elongated snout and main body compared to the individuals from the rest of the groups (Figure 4.11)

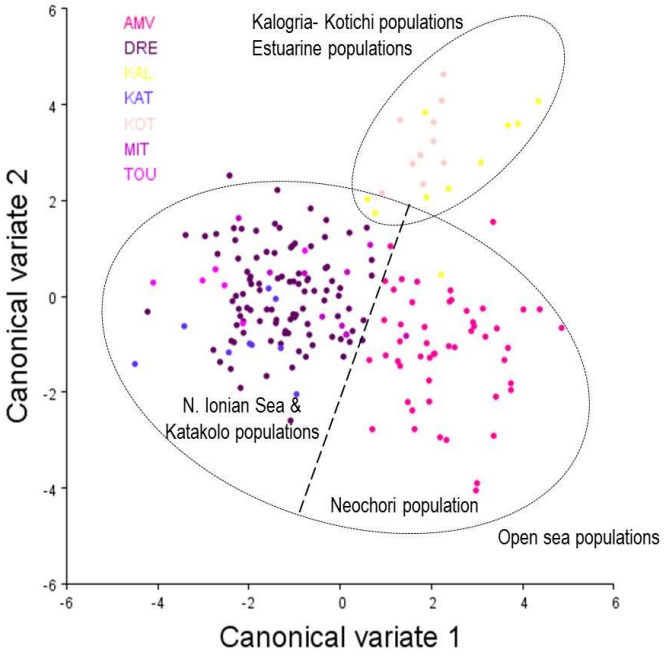


Figure 4.10. Scatter plot of the Canonical Variate scores of the morphometric structure analysis for Ionian Sea individuals of *S. abaster* species in the present study (stations codes after Table 4.2).
Εικόνα 4.10. Διάγραμμα διασποράς των τιμών της Ανάλυσης Κανονικών Συνιστωσών της ανάλυσης της μορφομετρικής δομής του είδους *S. abaster* από τους σταθμούς του Ιονίου Πελάγους (οι κωδικοί των σταθμών σύμφωνα με τον Πίνακα 4.2)

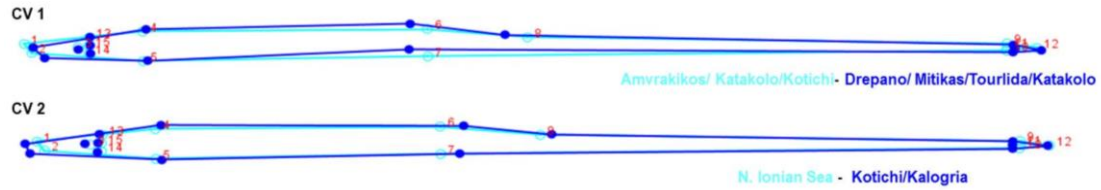


Figure 4.11. Observed shape changes associated with positive scores along the Canonical Variate Axes of the morphometric structure analysis for individuals of *S. abaster* in the Ionian Sea. The wireframes depict icons for deviations (dark blue line) from the corresponding mean shape for *S. abaster* species in the present study (light blue line).
Εικόνα 4.11. Παρατηρούμενη αλλαγή του σχήματος του σώματος με βάση τις θετικές τιμές του άξονα των Κανονικών Συνιστωσών της ανάλυσης της μορφομετρικής δομής του είδους *S. abaster* από τους σταθμούς του Ιονίου Πελάγους. Το πλέγμα απεικονίζει την απόκλιση (σκούρα μπλε γραμμή) από το σχήμα αναφοράς του είδους *S. abaster* κατά την παρούσα μελέτη (ανοιχτή μπλε γραμμή).

For the population of the Aegean Sea, nine CVs were extracted from the Canonical Variate Analysis. The first three explained 80.5 % the among group total variance (CV 1: 43.9%; CV 2: 25.3%; CV 3: 12.4%; CV 4: 11.3%). Mahalanobis and Procrustes distances (Table 4.13.c), as well as, the ordination of individual scores showed a considerable difference between stations defining three distinct groups: i) N.W. and C. Aegean Sea, ii) N.E. Aegean Sea and iii) S. Aegean Sea. The first two groups presented high negative scores along CV1 and were separated from the third (positive scores). N.E. Aegean Sea group had negative scores along the CV 2 and was separated from N.W. and C. Aegean Sea (positive scores) (Figure 4.12). The shape changes associated with the CV axes showed that: i) individuals from the N.E. Aegean group had larger head, smaller main body and slender trunk compared to N.W. and C. Aegean Sea ii) individuals from the S. Aegean Sea group had a more elongated snout and main body compared to N. Aegean Sea (Figure 4.13)

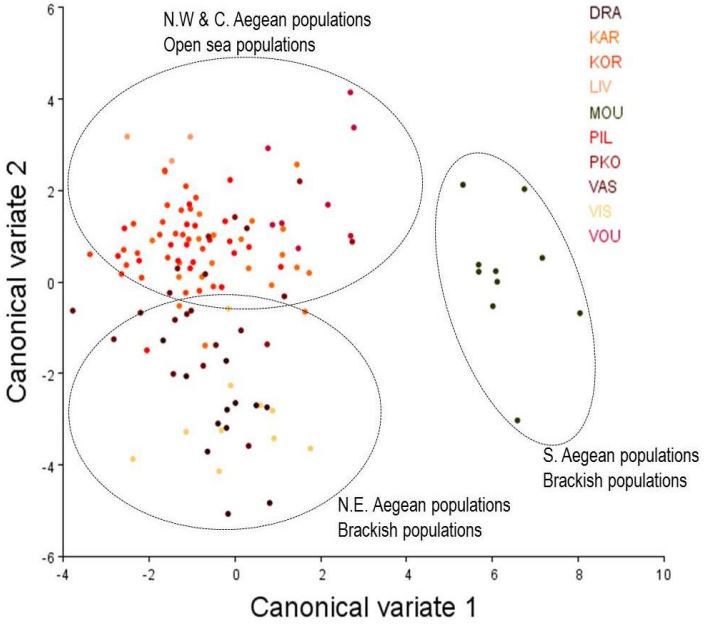


Figure 4.12. Scatter plot of the Canonical Variate scores of the morphometric structure analysis for individuals of *S. abaster* species from the Aegean Sea in the present study (stations codes after Table 4.2).
Εικόνα 4.12. Διάγραμμα διασποράς των τιμών της Ανάλυσης Κανονικών Συνιστωσών της ανάλυσης της μορφομετρικής δομής του είδους *S .abaster* από τους σταθμούς του Αιγαίου Πελάγους (οι κωδικοί των σταθμών σύμφωνα με τον Πίνακα 4.2).

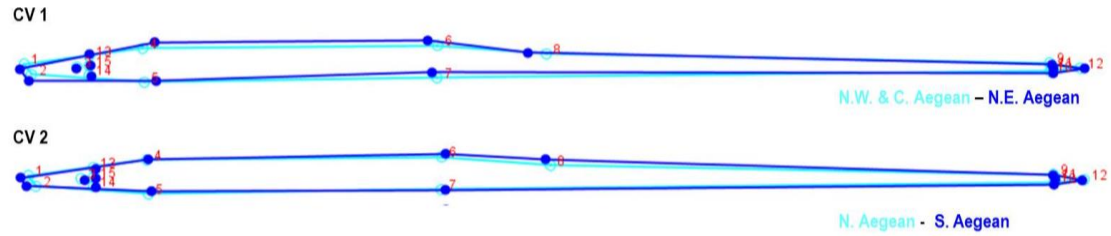


Figure 4.13. Observed shape changes associated with positive scores along the Canonical Variate Axes of the morphometric structure analysis for individuals of *S .abaster* from the Aegean Sea. The wireframes depict icons for deviations (dark blue line) from the corresponding mean shape for *S. abaster* species in the present study (light blue line).
Εικόνα 4.13. Παρατηρούμενη αλλαγή του σχήματος του σώματος με βάση τις θετικές τιμές του άξονα των Κανονικών Συνιστωσών της ανάλυσης της μορφομετρικής δομής του είδους *S .abaster* από τους σταθμούς του Αιγαίου Πελάγους. Το πλέγμα απεικονίζει την απόκλιση (σκούρα μπλε γραμμή) από το σχήμα αναφοράς του είδους *S. abaster* κατά την παρούσα μελέτη (ανοιχτή μπλε γραμμή).

4.3.3.2. *Syngnathus typhle* species

A total of 150 specimens of *S. typhle* species were collected along the sublittoral zone of Greece (n=124) and the Venice Lagoon (n=26) (Figure 4.1, Table 4.2). The PCA extracted 26 components (PCs). The first PC (PC 1) accounted for 39.2% of total variance, the second PC (PC 2) for 16.0 %, the third PC (PC 3) for 11.1% and the forth PC (PC 4) for 10.7%. These three PCs characterized, mainly, shape changes along the *x*-axis (PC 1 and 3) and to a smaller extend along the y-axis (PC 2 and 4). The rest of the PCs explain 23.1 % of total variance and described trivial aspects of intrapopulation shape variation. The PC scores were used as variables in the Canonical Variate Analysis to assess population structure.

Table 4.14. P- values for Mahalanobis and Procrustes distances morphometric structure analysis for individuals of *S. typhle* species from the Adriatic, Ionian and Aegean Sea, in the present study (stations codes after Table 3.2) .
Πίνακας 4.14. Τιμές της γεωμετρικής απόστασης κατά Mahalanobis και Procrustes κατά την ανάλυση της μορφομετρικής δομής του είδους του είδους *S. typhle* μεταξύ των πληθυσμών της Αδριατικής Θάλασσας, του Ιονίου και του Αιγαίου Πελάγους, της παρούσας μελέτης (κωδικοί των σταθμών σύμφωνα με τον Πίνακα 3.2).

	Mahalanobis		**Procrustes**	
	ADR	AEG	ADR	AEG
AEG	2.8478*		0.0103*	
ION	4.3211*	3.055*	0.0162*	0.0113*

* Statistical significant differences, P <0.05; * Στατιστικά σημαντικές διαφορές, P <0.05.

More specifically, the morphometric structure of *S. typhle* species was assessed by Canonical Variate Analysis (CVA) of partial warps and uniform components. Two Canonical Variates (CVs) were extracted (CV 1:87.3% and CV 2: 12.7%). Values of Mahalanobis and Procrustes distances revealed a strong morphological differentiation between individuals from the Ionian, Aegean and the Adriatic Seas (Table 4.14). Taking this into account and the ordination of individual scores, three distinct groups were revealed: i) Aegean Sea, ii) Ionian Sea and iii) Adriatic Sea (Venice Lagoon). CV1 separated the Ionian group from Adriatic and Aegean, while CV2 the Adriatic group from the Aegean (Figure 4.14). The shape changes associated with the CV axes showed that: i) individuals from the Ionian Sea had a shorter trunk and slender head compared to the Aegean and Adriatic, ii) individuals from the Adriatic Sea had a more elongated and curved body than the Aegean (Figure 4.14).

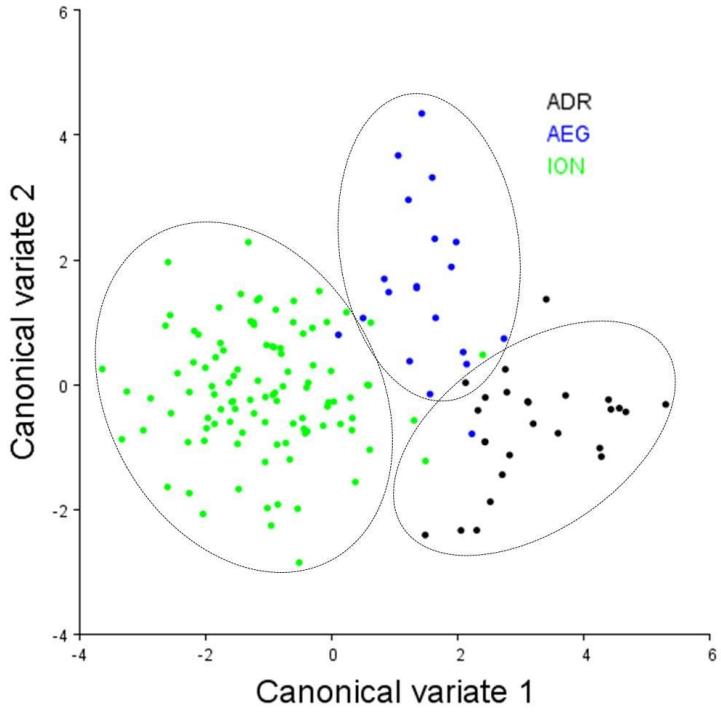


Figure 4.14. Scatter plot of the Canonical Variate scores of the morphometric structure analysis for individuals of *S. typhle* species from the Adriatic, Ionian and Aegean Sea in the present study (stations codes after Table 4.2).

Εικόνα 4.14. Διάγραμμα διασποράς των τιμών της Ανάλυσης Κανονικών Συνιστωσών της ανάλυσης της μορφομετρικής δομής του είδους *S . typhle* από τους σταθμούς της Αδριατική Θάλασσας, του Ιονίου και του Αιγαίου Πελάγους (οι κωδικοί των σταθμών σύμφωνα με τον Πίνακα 4.2).

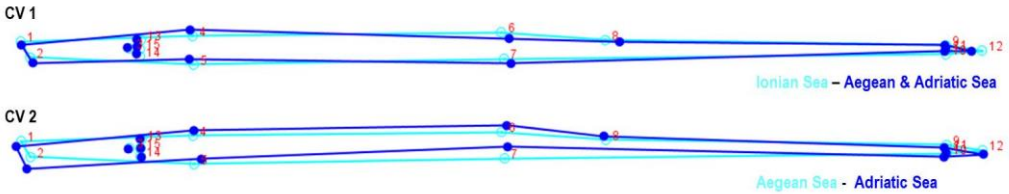


Figure 4.15. Observed shape changes associated with positive scores along the Canonical Variate Axes of the morphometric structure analysis for individuals of *S. typhle* from the Adriatic, Ionian and Aegean Sea. The wireframes depict icons for deviations (dark blue line) from the corresponding mean shape for *S. typhle* species in the present study (light blue line).

Εικόνα 4.15. Παρατηρούμενη αλλαγή του σχήματος του σώματος με βάση τις θετικές τιμές του άξονα των Κανονικών Συνιστωσών της ανάλυσης της ανάλυσης της μορφομετρικής δομής του είδους *S . typhle* από τους σταθμούς της Αδριατικής Θάλασσας, του Ιονίου και του Αιγαίου Πελάγους. Το πλέγμα απεικονίζει την απόκλιση (σκούρα μπλε γραμμή) από το σχήμα αναφοράς του είδους *S. typhle* κατά την παρούσα μελέτη (ανοιχτή μπλε γραμμή).

4.4. DISCUSSION

Studies of phenotypic variation play an important role in species identification. Sibling species are expected to show higher morphological similarity than remote ones. However, morphometric approaches are able to detect morphological differences even among congeneric or close related species (Campbell and Dickinson 1990; Moraes et al. 1994; Dhuyvetter et al. 2007). In the present study, multivariate techniques (PCA, DFA and CVA) seem to be an effective tool for distinguishing between and within *S. abaster* and *S. typhle* species, based on the suggested landmark morphometric protocol. The established protocol combines knowledge of previous morphometric works on syngnathids (e.g. Dawson 1986; Lourie et al. 1999; Thangaraj and Lipton 2011; Leysen et al. 2011; Mwale et al. 2013) and geometric morphometric techniques (Zelditch et al. 2004) providing a common ground for morphological comparison and species identification.

4.4.1. Morphological comparison of *Syngnathus abaster* and *Syngnathus typhle* species

Morphological comparison of *S. abaster* and *S. typhle* species indicated that the two species are completely separated. No intermediate morphotypes were observed, ruling out the possibility of hybridization. This outcome is in accordance with the species molecular based phylogenetic studies (Hablutzel 2009, Chapter 3 of the present thesis).

According to the Canonical Variate Analysis, *S. typhle* species had a more elongated snout and main body, whereas its trunk was shorter compared to *S. abaster*. The difference of the main body and the trunk between the two species was also supported by meristic characters. *S. typhle* had more rings in the main body (predorsal) and less in the trunk (underdorsal and postdorsal) compared to *S. abaster*. Lindsey (1975) proposed that main body rings are under selection in the genus *Syngnathus*, i.e. larger species have more vertebrae. As shown by Dawson (1986) *S. typhle* is a larger species compared to *S. abaster*. So, Lindsey's general pattern seems to apply in both species.

Moreover, the observed snout shape changes could probably be attributed to variations in the foraging ecology of the two species (Keast and Webb 1966; Wainwright and Richard 1995). Species with shorter snouts generally feed on smaller and less mobile preys, while pipefish species with larger snouts can feed on larger and faster preys (Franzoi et al. 2004; Oliveira et al. 2007; Roos et al. 2009; Leysen et al. 2011; Van Wassenbergh et al. 2011). In particular, *S. typhle* specimens had a longer snout compared to *S. abaster*. This morphometric dichotomy is in accordance with the feeding strategy of the two species. As the mouth opens its lateral walls expand, increasing the volume of the snout. This increase as well as the quantity of the inhaled water determines the preying ability of each species. For instance the long snout of *S. typhle* can capture relatively fast moving and large sized pelagic preys. In fact, as *S. typhle* grows its diet changes progressively from Copepods to Mysidacea and later to small size Caridea and Gobiidae (Oliveira et al. 2007). On the contrary, *S. abaster* demonstrates moderate diet succession with a preference on little prey hidden in the vegetation (Franzoi et al. 2004).

Similar correlation of snout morphology and feeding ecology in sympatric syngnathids has been observed in *Syngnathus taenionotus* and *S. abaster*. *S. taenionotus* had a longer and terminally cylindrical snout which enables it to capture fast moving preys, compared to the short- snouted *S. abaster* species (Franzoi et al. 1993). Also the sympatrical occurring syngnathids *Phyllopteryx taeniolatus*, *Vanacampus poecilolaemus*, *Pugnaso curtirostris* and *Histiogamphelus cristatus* exhibited a similar pattern. In particular, the first two species feed on relatively mobile prey and have longer snouts compared to the last two species, which feed on slow moving preys and have shorter snouts.

Therefore, phenotypic variation between *S. abaster* and *S. typhle* species could be attributed not only on genetic divergence but also on different ecological niches. These niches formed strong ecomorphological patterns that resulted in distinct phenotypes among the two species. Morphometric variability among other sympatric and congeneric species of different organisms, such as members of the subfamily of Maloideae (Campbell and Dickinson 1990), the oak species *Quercus virginiana* and *Querus geminate* (Cavender-Bares and Pahlich 2009) and the carabid species *Pogonus littoralis* and *Pogonus chalceus* (Dhuyvetter et al. 2007), reinforce the findings of the present study.

4.4.2. Sexual dimorphism of *Syngnathus abaster* and *Syngnathus typhle* species.

In nature, there are three principal forces that drive sexual dimorphism (Berglund et al. 1986). First of all, the interaction of natural selection and fecundity may act differently on each sex. For instance, in many species fecundity increases proportionally to body size only in female specimens (Wootton 1979; Tollestrup 1982; Berglund et al. 1986). The second force is the interaction of natural selection and food competition between the sexes that leads to higher feeding rates of the larger sex (Slatkin 1984; Olveira et al. 2007). Finally, sexual selection can lead to sexual dimorphism. Usually, the sex that competes harder for mates is more sexually dimorphic that the choosy one (e.g. Trivers 1972; Wade and Arnold 1980; Andersson 1982; Berglund et al. 1986).

Syngnathus species are susceptible to sexual dimorphism, with females being usually larger and brighter than males (Berglund et al. 1986). This was also the case of *S. abaster* and *S. typhle* species from Drepano and Neochori stations. Females had a more elongated and thicker snout and main body and at the same time a more slender trunk compared to males. This comes as no surprise, as *Syngnathu*s species are Urophori i.e. carry their embryos in the brooding pooch which is located in their trunk (Herald 1959). So, males need more space to carry their embryos resulting in a thicker trunk than females. On the contrary females have larger main body in order to produce and store as many eggs as possible.

The difference in the snout shape between males and females could be related to different feeding habits of both sexes and/or the existence of sexual dimorphism. Particularly Svensson (1988) showed that females of *S. typhle* species had higher feeding rates, than males. On the contrary pregnant males reduce their preying capacity in order to avoid predation and ensure the survival of their offspring. Therefore, as females of *S. typhle* feed on faster and larger preys they also need larger mouth (Svensson 1988). Even though feeding ecology of both sexes is not established in *S. abaster* species, judging by the difference in snout morphology of males and females, a similar pattern as *S. typhle* species must be followed.

So, sexually dimorphic patterns are obvious and could be related to anatomical structures and feeding ecology. At this point is has to be mentioned that the observed sexual dimorphism was not correlated with the length of the species but with their overall shape.

4.4.3. Morphometric pattern of *Syngnathus abaster* and *Syngnathus typhle* species along the coastline of Greece.

The phenotypic variation among individuals of *S. abaster* and *S. typhle* showed that both species form three morphometricaly distinct populations: i) Aegean ii) Ionian and iii) Adriatic Sea. As it is already known, these three Seas form distinct biogeographical regions with different environmental and geological conditions (Bianchi 2007; Coll et al. 2010). Phenotypic variability among these Seas has been recorded for other marine organisms besides the two studied species. For instance, Mediterranean *Diamysis* species differ morphologically among the Ionian and the Adriatic Sea (Ariani and Wittmann 2000), while anchovy, sardine and red mullet form distinct populations in the Ionian and the Aegean Sea (Spanakis et al. 1989, Mamouris et al. 1998 a).

These results were expected for *S. abaster* species as molecular analysis had indicated that populations from the Ionian and Aegean Sea formed two distinct clades. Also, at a broader scale, the isolation by distance pattern indicates that the populations from the Greek coastline and the Adriatic Sea must be genetically distinct. Therefore, the morphological pattern is in accordance with the genetic population structure.

The morphometric pattern of *S. typhle* revealed phenotypic differentiation between the Ionian, Aegean and Adriatic Seas. The difference between the Greek populations and the Adriatic Sea was expected. As shown in the phylogenetic analysis (Chapter 3) Adriatic and Ionian-Aegean Sea are distinct populations. Therefore, the difference in their genetic structure could impact on species morphology, probably resulting in distinct phenotypic patterns. However, within the Greek coastline the species genetic structure does not follow a specific geographic pattern (Chapter 3). Therefore, the two morphologically distinct clades could not be correlated to genetic background (at least not for the studied loci).

At this point it has to be stated that morphometric characters are only partially genetically determined (Griffiths et al. 2010) and are also under the influence of natural selection (Arnold 1983; Lande and Arnold 1983; James 1983). In fish species morphological variation may result from phenotypic plasticity in response to trophic and environmental conditions prevailing in each area (Corti et al. 1996; Clabaut et al. 2007). Therefore, the above noticed morphological pattern could be a form of local adaptation to the variable environmental and physicochemical conditions of Ionian and Aegean Seas (Coll et al. 2010).

Besides the variability at a broader- E. Mediterranean scale (Adriatic, Ionian and Aegean Sea), morphometric analysis of *S. abaster* species showed distinct clades within the populations of Ionian and Aegean Sea. More specifically, as already mentioned Ionian populations were separated in three groups: i) N. Ionian Sea- Katakolo, ii) Kotichi-Kalogria and iii) Amvrakikos Gulf. This grouping is incongruent with the results of the species genetic structure. In particular the species Ionian Sea's haplotypes form one cluster (Figures 2.8, 2.13). However, they corresponded to populations from different types of

ecosystems. Kotichi and Kalogria populations were the only ones from estuarine ecosystems in the Ionian Sea sample. The rest of the populations were sampled from coastal marine ecosystems. From the latter, Neochori population was the only one from a protected Gulf. Therefore, it is obvious that the three morphological groups coincide with three different types of ecosystems: i) open sea marine, ii) estuarine and iii) semi-enclosed protected marine.

Within the Aegean populations of *S. abaster* species, three distinct clades were, also, formed: i) N.W. and C. Aegean Sea, ii) N.E. Aegean Sea and iii) S. Aegean populations. These clades do not only have a biogeographical meaning but also an ecological one. The populations of the N.W. and C. Aegean Sea are all from open sea ecosystems, while the rest of the populations were sampled from brackish waters.

Therefore our original hypothesis that *S. abaster* species would form phenotypically distinct populations seems to be verified, even though genetic and morphometric population structure was similar but not identical. In the case of *S. typhle* species the distinct morphological clades contradict the significant yet unrelated to geographic distances genetic structure. Therefore, it is suggested that both genetic population structure and local adaptations- as response to different habitat conditions- shaped the phenotypic profile of *S. abaster* and *S. typhle* species.

Chapter 5. Male pregnancy and genetic mating system of *Syngnathus abaster* and *Syngnathus typhle* species in the N. Ionian Sea

5.1. INTRODUCTION

Male pregnancy and mating system are two of the most interesting characteristics of syngnathids biology and *S. abaster* and *S. typhle* are the most well-studied European pipefishes concerning these traits (e.g. Berglund et al. 1988; Vincent et al. 1994; Silva et al. 2006a, Rispoli and Wilson 2008; Hubner et al. 2013). The inverted brood pouch of *S. abaster* is a complex closed structure, located in the tail of the males' body. It constitutes of two skin folds that are in contact with their free edges and cover the eggs (Carcupino 1997; 2002). The formation of a "pseudo-placenta" and the high concentration of mitochondrial-rich (MR) and chloride cells suggest that the pouch osmoregulates and nourish the developing embryos (Carcupino 1997).

Male pregnancy lasts from three to four weeks, at ambient temperature and even fluctuations of two or three degrees can alter the duration of the pregnancy (24–32 pregnancy days at 18–19 ^{o}C, while only 21 days at 21–22 ^{o}C) (Silva et al 2006a). According to Silva et al. (2006a), regardless of the span of the pregnancy, during the embryonic and larval development the ontogenetic events occur as follows: a) blastula stage, b) epiboly, c) embryonic shield formation, d) cephalic and caudal dilatation, e) optic vesicles formation, f) notochord and neural tube differentiation, g) beginning of somite formation, h) crystalline lens shape, i) ocular pigmentation, j) tail region free from yolk, k) beginning of melanogenesis, l) fin differentiation, m) heart beats and visible blood vessels, n) embryo motility, o) mouth apparatus development, p) fin rays development, q) dermal plates, r) coloration, s) hatch from egg envelope, t) release from marsupium (Figure 5.1). The released embryos (from 24 to 37 specimens, Silva et al. 2006a, Cunha 2012, Hubner et al. 2013) are fully formed juveniles, resembling adults. After parturition, newborns immediately assume a benthic distribution (Silva et al. 2006a).

Figure 5.1. Developmental stages of *S. abaster* species as described in brooding males from Ria de Aveiro lagoon (Portugal): (a) 3 days, (b) 7 days, (c) 13 days, (d) 17days, (e) 19 days, (f) 21 days, (g) newborn head detail and (h) newborn juveniles. Scale bars 1 mm (after Silva et al. 2006a).
Εικόνα 5.1 Εμβρυικά στάδια ανάπτυξης του είδους *S. abaster*, όπως περιγράφηκαν από κυοφορούντα αρσενικά άτομα από τη λιμνοθάλασσα Ria de Aveiro (Πορτογαλία): (a) 3^{ης} ημέρας, (b) 7 ^{ης} ημέρας, (c) 13 ^{ης} ημέρας (d) 17 ^{ης} ημέρας, (e) 19 ^{ης} ημέρας, (f) 21 ^{ης} ημέρας, (g) λεπτομέρεια της περιοχής της κεφαλής και (h) νεογέννητο νεαρό άτομο. Κλίμακα 1 mm (σύμφωνα με Silva και συν. 2006a).

The reproductive behavior and the social mating system of *S. abaster* species under reared conditions have been extensively described by Silva et al. (2006b; 2008; 2010). Prior to reproduction both sexes engage in courtship and mating rituals accompanied by flickering and parallel movements above the vegetation (Figure 5.2). Secondary sexual traits evolve in both sexes particularly in the abdominal region. Males were observed to mate with a maximum of three females, while females were also observed to spawn with more than one male. Therefore, the social mating system is characterized as polygynandrous (Silva et al. 2006b). The pieces in the puzzle of *S. abaster* mating system were put together when Cunha (2012) and Hubner et al. (2013) described the species genetic mating system. In a microsatellite based parentage analysis they showed that both males and females mate multiple times. Therefore, they confirmed that *S. abaster* is a polygynandrous species.

Despite the species wide distributional range, data on social and genetic mating system are available only from the above mentioned brackish populations in Portugal (Cunha 2012; Hubner et al. 2013). However, as proven in other syngnathid species the mating system is strongly affected by temperature (Ahnesjo 2008), sexual selection (Rispoli and Wilson 2008; Mobley and Jones 2007; 2009; Wilson 2009; Monteiro and Lyons 2012) demography (Mobley and Jones 2007; Rispoli and Wilson 2008) and/or habitat (Mobley and Jones 2009). Therefore, data from only one region can under- or overestimate the level of polygamy or obscure aspects of the mating system.

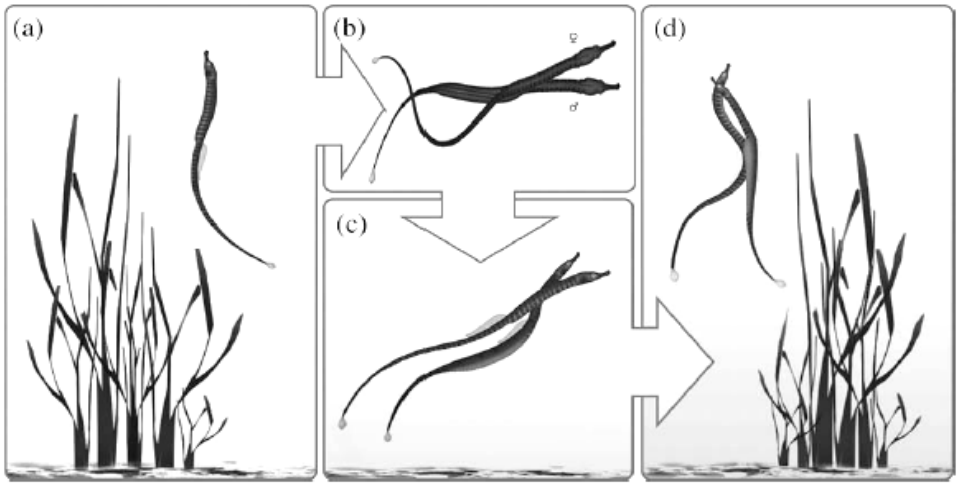


Figure 5.2. Overview of the main stages of courtship and mating behavior of *S. abaster* species: (a) vertical swimming, (b) crossing, (c) parallel swimming and (d) spawning (after Silva et al. 2006b).
Εικόνα 5.2. Περιγραφή των κύριων σταδίων της ερωτοτροπίας του είδους *S. abaster*: (a) παράλληλη κίνηση στη στήλη του νερού, (b) πλέξιμο των σωμάτων, (c) παράλληλη κίνηση κάθετα στη στήλη του νερού and (d) γονιμοποίηση (σύμφωνα με Silva και συν. 2006b).

Extensive laboratory and field studies have broadened the horizons of male pregnancy, reproductive ecology and mating system of ***S. typhle*** (e.g. Berglund et al. 1988; Vincent et al. 1994; 1995; Jones et al. 1999; Rispoli and Wilson 2008; Goncalves et al. 2010). The brood pouch of *S. typhle* is characterized as "inverted" with a similar structure and organization as *S. abaster* (Herald 1959; Berglund 1989; Vincent et al.1994). The brood pouch can accommodate from 20 to 250 embryos with an average range of 23±65 (Berglund et al. 1986; Vincent et al. 1995; Jones et al. 1999). During male pregnancy there is a positive correlation between male body size and clutch size (i.e. number of offspring that the male pouch can accommodate), with larger males carrying larger embryos than smaller ones (Berglund et al. 1986). At the same time, the average number of eggs transferred from a female to each male per spawning incident, is positively correlated with female body size (Berglund et al. 1986).

The embryonic and larval development of the species remains an undiscovered territory. However, Sommer et al. (2012) proposed that differences in the developmental stages may result from variations in the level of brood pouch complexity. Therefore, species with the same pouch structure- such as *S. typhle* and *S. abaster*- could undergo similar- if not identical- embryonic and larval stages (Figure 4.1), even if the duration of the stages differs among species. However, this theory is an indication that needs to be tested and verified in a wider spectrum of species before being confirmed.

The mating system of *S. typhle* was one of the first to be explored in the Syngnathidae family. Both laboratory and field observations indicate that, prior to mating, males and females are engaged into an elaborate courtship behavior and dance -similar to *S. abaster* species- that ultimately determines the success of the mating (Vincent et al. 1995). Both sexes are observed to mate multiply in one breeding cycle i.e. one male carries eggs of more than one female, while one female allocates her eggs in two or more males (Berglund et al. 1988, Vincent 1994; 1995). These observations were the first evidence of polygynandry. Larger females are usually the ones to allocate their eggs in more than one male compared to smaller. This is done probably in order to minimize the variance on offspring number and reduce sib-sib competition among their large eggs (Berglund et al. 1988)

Observations based on field and laboratory studies of *S. typhle* mating system are also supported by genetic data (Jones et al. 1999; Rispoli and Wilson 2008). Microsatellite-based parentage analysis indicated that males mate with multiple females, while the same female may sire embryos in the brood pouch of more than one male. Thus, the genetic mating system of *S. typhle* is confirmed as polygynandrous. An interesting finding is that the degree of male polygamy is positively correlated with sexual size dimorphism suggesting that when males can brood more embryos, females are also able to produce more eggs (Rispoli and Wilson 2008).

The above overview of male pregnancy reveals that even though many aspects have been unraveled, there are still unexplored territories, such as the behavior of brooding

males during gestation and the actual process of the parturition. Even though less studied, these are important aspects of the reproductive biology and ethology of the two species. More specifically, the structure and the content of the pouch change during pregnancy as: a) changes in the epithelium tissue occur in order to osmoregulate the embryos, b) the tissue of pseudo-placenta forms, c) embryos hatch from their eggs and develop to fully shaped juveniles ready to be released and d) the two skin folds of the pouch that cover the eggs open and release the offspring (Carcupino et al. 1997; 2002; Silva et al 2006a; Sommer et al. 2012). In order to go through with these changes- especially the last two- the volume and the shape of the pouch cannot stay the same and has to change. Combined with the fact that the brood pouch of the two species is semi-transparent (more or less), two questions arise, the answer of which was the first goal of the present chapter: How do these structural changes and offspring developmental stages depict on pouch morphology? If the impact is visible could it be used as an indirect estimator of the embryonic and larval developmental stages? Given that the brood pouch of the two species is semi-transparent it was hypothesized that, as embryos hatch from their eggs and develop into fully formed juveniles, their body pigmentation would be visible and could potentially alter the color of the pouch. If this is the case, black colored pouches could be a sign of advanced pregnancy while light colored a sign of early developmental stages.

Moreover, to our knowledge so far, the actual procedure of parturition has only been fully described in the seaweed pipefish *Syngnathus schlegeli* and in the opossum pipefish *Microphis brachyurus* (Watanabe and Watanabe 2002; Frias-Torres 2004). Silva et al. (2006a) refer briefly to some aspects of *S. abaster* offspring release too. In these three studies parturition is described as an intense procedure, accompanied with strong vibrations and contractions of the body. The second goal of this chapter was to describe the proses of parturition and examine how two species with different vertical position but same pouch type go through this ordeal. The two studied species assume different vertical positions, despite the fact that they have the same brood pouch type. *S. abaster* is a benthic species with a bottom dwelling preference, while *S. typhle* mostly occupies the upper part of the canopy and the water column in a more vertical posture, aligning the body with the artificial leaves (Malavasi et al. 2007). Taking that aspect into account, it was hypothesized that the two species would retain their spatial segregation throughout pregnancy, and during parturition they would vibrate along their vertical axis.

Furthermore, Silva et al. (2006a) revealed that *S. abaster* offspring are released from the brood pouch fully formed, are miniatures of adult specimens and acquire a benthic behavior right after birth. Given the resemblance of newborns to adult specimens, could distinct features between adults *S. abaster* and *S. typhle* species (size, shape, anatomical traits and vertical position) be visible in offspring too? The answer to that question was the third goal of the present chapter. In order to address it, the total length and weight, morphometry, number of dorsal fin rays and vertical position of both species' offspring was examined and compared. If the newborns were actually miniatures of adult specimens larger sized newborns of *S. typhle* with more dorsal fin rays than *S. abaster*

were expected to be found, following the morphological pattern and the gesture of their parents.

As already mentioned, both species are considered polygynandrous (Jones et al. 1991; Rispoli and Wilson 2008; Cunha 2012; Hubner et al. 2013). This assumption is based on analysis of populations over a wide range of *S. typhle* distributional range (Jones et al. 1991; Rispoli and Wilson 2008), but only from Portugal populations as regards *S. abaster* (Cunha 2012; Hubner et al. 2013). However, existing studies on *S. typhle* and its congeneric *S. floridae* showed that the mating system and level of multiple mating may vary over space and time due to ecological reasons (e.g. temperature, predation, population density), sexual selection (e.g. sexual size dimorphism) and/or geographic isolation (Rispoli and Wilson 2008; Mobley and Jones 2007; 2009).

Also, molecular analysis (Chapter 3) revealed distinct clades of *S. abaster* between Ionian and Aegean Seas, while *S. typhle* exhibited isolation by distance pattern only at a European scale. Sexual size morphometric analysis of *S. abaster* and *S. typhle* revealed that females have a more elongated and thicker snout and main body and at the same time a more slender trunk compared to males (Chapter 4). However, the dimorphism was not translated into differences in the total length between males and females of the Ionian Sea (Chapter 2). The absence of sexual size dimorphism is in contrast with the so far studied populations of both species (Berglund et al. 1986; Vincent et al. 1995; Silva 2006b; Rispoli and Wilson 2008; Hubner 2013).

Under this perspective, the final goal of the present chapter was to describe the genetic mating system of *S. abaster* and *S. typhle* in the N. Ionian Sea. More specifically, it was examined if geographical isolation and the absence of sexual size dimorphism could impact the degree of multiple mating of both species. It was hypothesized that geographical isolation and absence of size dimorphism would affect mating behavior in both species compared to the populations across their distribution range that show signs- even moderate- of sexual selection.

5.2. MATERIALS AND METHODS

5.2.1. Sampling method

S. abaster and *S. typhle* individuals were collected with a hand-net (30 cm high and 40 cm long, mesh size of 2 mm) in Drepano station (for details see Chapter 2.2.1, 2.2.2.) in the first half of the reproductive period of 2012 (May-July). Sex was examined on the spot macroscopically by the presence (males) or absence (females or immature individuals) of the brood pouch. A representative sample of females and non-brooding males of both species (*S. abaster*, N: 34; *S. typhle*, N: 18) was stored at 80% alcohol solution in order to be used in the parentage analysis. These individuals constituted the sample of the wild population (WP). The rest of the sampled non- brooding males, females and immature individuals were released back to the sea. Pregnant males were kept in an open tank with sea water until the end of the sampling effort. Then, the degree of their pouch fullness was examined. Males with half full or empty pouches were returned to the sea, while those with full pouches were kept in the tank and transported in the laboratory. In total, seventeen and six males of *S. abaster* and *S. typhle* species, respectively, with full pouches were caught during the 3 months.

5.2.2. Fish maintenance

Pregnant males of *S. abaster* and *S. typhle* males were brought to the laboratory. Each male was placed in a separate 25 L aquarium and kept under natural light (Figure 5.3). A "pseudo substratum" was placed in the bottom of the aquarium. The pseudo-substratum consisted mainly of sand, gravel and plastic seagrass in order to mimic the original habitat where fish were caught and filter the water of the aquarium. Oxygen was supplied via air pumps. In order to avoid the 'gas bubble disease', common in pipefishes (Monteiro et al. 2002), aeration was performed in a cylindrical tube inside the tank. Salinity remained constant in 37 psu as water was partially renewed every second day with fresh water transferred from Drepano station. Fish were fed twice a day with a mixture of frozen zooplankton and artificial food pellets. From the seventeen captured males of *S. abaster* species, eleven were able to survive and give birth to viable offspring under reared conditions. During the same period half of the males of *S. typhle* died before giving birth (3 pregnant males survived).

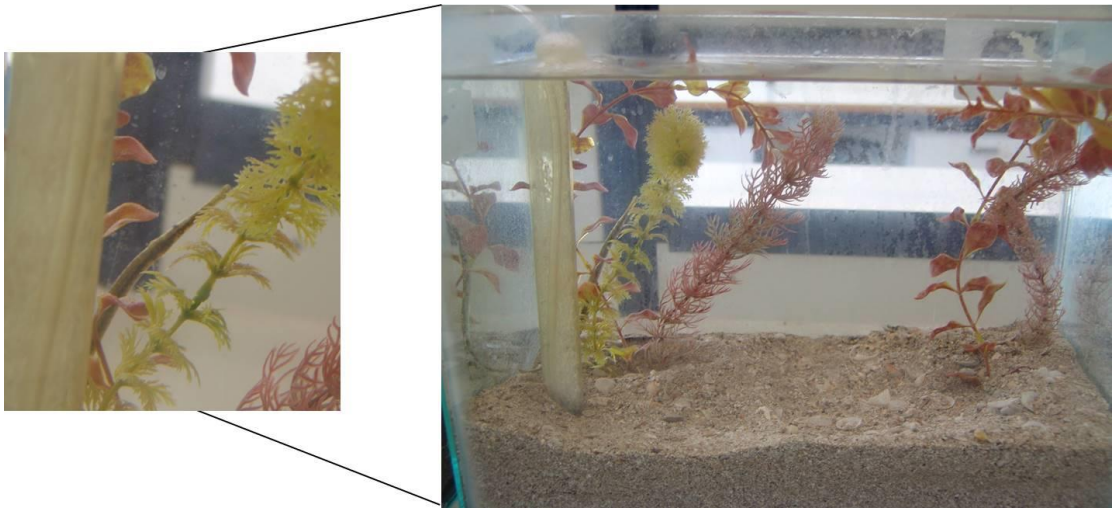


Figure 5.3 Brooding male of *S. typhle* species lying among plastic seagrass in the 25L aquarium with sandy- gravel substratum during the present study.
Εικόνα 5.3. Αρσενικό κυοφορούν άτομο του είδους *S. typhle* ανάμεσα στη τεχνητή βλάστηση του ενυδρείου (25L) με αμμώδη- χαλικώδη πυθμένα κατά τη διάρκεια της παρούσας μελέτης.

5.2.3. Data collection

Fish were kept in the aquarium until they released all the embryos of their brood pouch. The labor was recorded and notes were kept on each species behavior. Newborn juveniles were removed from the aquarium and put in ice. Once they were immobilized, they were separated according to their color and counted. In each individual the number of dorsal fin rays (NDFR) was counted and total length (TL) and total weight (TW) were measured to the nearest 0.1 mm and 0.001 gr, respectively. Each individual was stored at 80% alcohol solution in order to be used in parentage analysis. The father (adult male) was processed in the same way.

5.2.4. Statistical analysis

The total length, total weight and number of dorsal fin rays of the newborns and the father was compared between and among the two species and between and among each birth using the Kruskal-Wallis ANOVA non-parametric test (H) (Zar 1999) as the assumption of homogeneity was not met. The correlation of total length and weight of adult males with the number of released newborns was performed by Spearman's rank correlation coefficient (rho) (Zar 1999). Statistical analyses were performed with SPSS ver 21.0.

5.2.5. Parentage and genetic mating system analysis

5.2.5.1. Selected loci

In the present study four microsatellite loci -S. abas3, S.abas4, S.abas7 and S.abas9- were used to assess the genetic mating system and parentage analysis of *S. abaster* and *S. typhle* species (Table 5.1.). These loci have been previously isolated and characterized for *S. abaster* and successfully cross-species amplified in *S. typhle* species by Diekmann et al. (2009). They have been, already, successfully used in genetic mating system and parentage analysis of *S. abaster* species from Ria Fermossa Lagoon (Cunha 2012; Hubner et al. 2013).

5.2.5.2. DNA extraction

Genomic DNA from all specimens was extracted from tail muscle tissue after the modified phenol-chloroform extraction protocol (Sambrook and Russel 2001). The procedure followed is described in detail in Chapter 3 of the present thesis. The amount of extracted DNA was quantified by loading 5 μl of each extraction on a 1 % agarose gel stained with ethidium bromide.

5.2.5.3 Polymerase Chain Reactions

PCRs were performed in 10 μl volume containing 5U KAPA Taq ReadyMix (KAPABIOSYSTEMS), 25 mM $MgCl_2$, 100 pmol of reversed primer, 100 pmol of IRD800[3]-labeled forward primer and 100 ng DNA. Negative (1 μl ddH_2O instead of DNA) and positive (100 ng DNA of an individual whose loci had already been successfully amplified) controls were included in all reactions. PCR reactions were performed on an MJ Research PTC- 200 gradient cycler under the conditions shown in Table (5.2). To ensure amplification, PCR products were visualized in 1.5% agarose gels.

5.2.5.4. Genotyping of samples[4]

Pregnant males, a representative subsample of their offspring and wild population individuals were genotyped at the four loci. As already mentioned, offspring were stored according to their coloration. Within each brood, approximately five individuals per color were genotyped.

[3] IRD800 is a heptamethine cyanine dye absorbing and fluorescing in the near infrared region of the spectrum (800 nm).

[4] Genotyping procedure described after the manual of Li-COR 4200 DNA analyzer.

Table 5.1. Primer sequences and allele sizes of the four microsatellite loci that were used in the parentage analysis of *S. abaster* and *S. typhle* species, in the present study (*F*, forward primer; *R*, reverse primer; *IRD800*, primer label; *size range*, PCR product size at each locus).
Πίνακας 5.1. Ακολουθίες ολιγονουκλεοτιδικών εκκινητών και μέγεθος αλληλομόρφων των τεσσάρων μικροδορυφορικών τόπων που χρησιμοποιήθηκαν στην ανάλυση πατρότητας των ειδών *S. abaster* και *S. typhle* στην παρούσας εργασίας (*F*, εκκινητής νοηματικού κλώνου; *R*, εκκινητής μη νοηματικού κλώνου; *IRD800*, χρωστική για τη σήμανση του εκκινητή; *size range*, το μέγεθος του προϊόντος της αλυσιδωτής αντίδρασης πολυμεράσης κάθε τόπου)

Locus	Primer sequence (5'-> 3')	Repeats	Size range (bp)
S.abas3	F: IRD800-TTCCCCCTAGGACCAATAAAGTATCT R: TGAGAGTGGTTGCCTCCAGC	$(ATCT)_{37}$	166-294
S.abas4	F: IRD800-ACAAAATGCAAGTGATCCTGTGTAGG R: TGGTGTGGTGGAACTGAATGACG	$(TCTA)_{38}$	193-497
S.abas7	F: IRD800-CGATGTGCGAGACCTGTTGCG R: AAAGAGGCGGAGCTTGTGTAAGGA	$(GATA)_{33}$	198-498
S.abas9	F: IRD800-TGATTTGGAATGACACGGGTGGTTTG R: TCGTTTTGTGTGCACCGAGTGTT	$(ATAG)_{33}$	192-424

Table 5.2. PCR conditions for the microsatellite loci S.abas3, 4, 7 and 9 used in the preset study
Πίνακας 5.2. Συνθήκες που ακολουθήθηκαν στις αντιδράσεις PCR για τους μικροδορυφορικούς τόπους S.abas3, 4, 7 and 9 κατά τη παρούσα μελέτη.

Steps	Loci	
	S.abas3, 4 and 7	**S.abas 9**
Step1. Initial denaturation	94 °C for 360 sec	94 °C for 360 sec
Step2. Denaturation	95 °C for 30 sec	95 °C for 30 sec
Step3. Annealing temperature	64 °C for 30 sec	67 °C for 30 sec
Step4. Extension	72 °C for 60 sec	72 °C for 30 sec
Step5.	repetition of steps 2-4 for 37 cycles	repetition of steps 2-4 for 37 cycles
Step6. Final extension	72 °C for 20 min	72 °C for 20 min

Genotyping was performed on a semi-automated Li-COR 4200 DNA analyzer. The LI-COR System used infrared (IR) fluorescence to detect DNA. DNA polymerase incorporated the used IRD800-labeled primer into a set of chain-terminated fragments. These fragments separated according to size on the 6.5% (*LI-COR*® KB Plus™) acrylamide gel (length*width: 25*0.25 (cm*mm). TBE 1X was used as loading buffer. *LI-COR*® Size Standard IRDYE™ 800 (50-350 bp) was used as molecular weight ladder. The ladder was composed of 15 IRD-labeled DNA fragments with equal banding intensities (350, 325, 300, 255, 230, 204, 200, 175, 145, 120, 105, 100, 94, 75 and 50 bp) in 90% formamide solution with bromophenol blue.

A solid-state laser diode excites the infrared dye on the DNA fragments as they migrate past the detector window. A focusing fluorescence microscope- containing a solid-state silicon avalanche photodiode- scans the whole gel back and forth collecting data in real time. The raw image data are displayed in an autoradiogram-like format on the computer screen as a series of bands (Figure 5.4). Genotyping was conducted manually in SagaGT software by two researchers working separately. The results were afterwards cross-checked to validate the genotypes.

One or two days before the electrophoresis, PCR products were diluted in formamide solution constituting of formamide and loading buffer (glycerol 50%: bromophenol blue 0.1%: methylene blue 0.1% = 8V:1V: 1V) in a ratio of 10:1. Dilution depended on the concentration of PCR products and it ranged from 1:3 (concentration < 10 ng/µl) to 1:50 (concentration > 20 ng/µl). Prior to electrophoresis PCR products were denaturated at 95 ^{o}C.

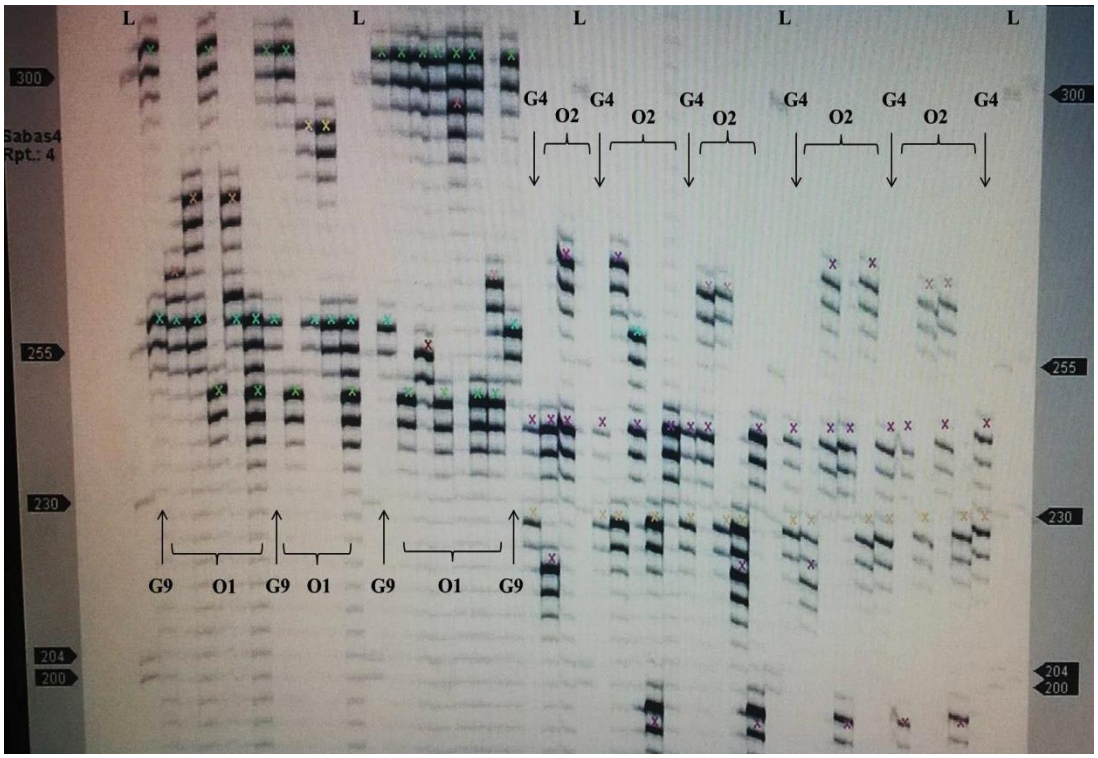


Figure 5.4. Genotype pattern of microsatellite locus S.abas4 in polyacrylamide gel in the present study (*M*, molecular weight marker with shown bands of 200, 204, 230, 255 and 300 bp; *G9* and *G4*, fathers' genotypes; *O1*, genotypes of G9 offspring; *O2*, genotypes of G4 offspring; *x*, genotyped alleles; *the color of x* differs according to the allele's size, same coloration in x indicates alleles of the same size).

Εικόνα 5.4. Πρότυπα γενοτύπων του μικροδορυφορικού τόπου S.abas4 της παρούσας μελέτης (*M*, μάρτυρας μοριακού βάρους με εμφανείς ζώνες στα 200, 204, 230, 255 and 300 ζ.β.; *G9* and *G4*, γενότυποι δύο αρσενικών γεννητόρων; *O1*, γενότυποι των απογόνων του G9; *O2*, γενότυποι των απογόνων του G4; *x*, γενοτυπημένα αλληλόμορφα; *το χρώμα του x* διαφέρει με το μέγεθος του αλληλομόρφου, το ίδιο χρώμα εμφανίζεται σε αλληλόμορφα του ίδιου μεγέθους).

5.2.5.5. Microsatellite analysis

After genotyping, allele frequencies, number of alleles per locus, observed (*H*o) and expected (*H*e) heterozygosity, Hardy-Weinberg equilibrium, Polymorphic Information Content (PIC) and null alleles frequency were calculated in each locus using Cervus 3.0 software (Kalinowski et al. 2007). Hardy-Weinberg equilibrium was tested by the exact test using the Markov chain method. The PIC value takes into consideration both the number of alleles and their relative frequencies. Compared with the index of heterozygosity, it provides additional information regarding the relative frequencies of alleles at a marker locus (high polymorphism if PIC value > 0.5) (Botstein et al. 1980).

5.2.5.6. Parentage analysis and genetic mating system

Microsatellites are usually co-dominant and therefore, each genotyped embryo carries a record of both maternal and paternal alleles. Brood pouch ensures male syngnathids for the paternity of their offspring. Therefore, the father's allele is always known. The second unknown allele is inherited by the mother and can be deduced by subtraction. Colony software (Jones and Wang 2009) was used to reconstruct the mother's genotypes from arrays of half- or full- siblings.

The total length and number of dorsal fin rays of offspring from different mothers were compared using the Kruskal-Wallis ANOVA non-parametric test (H) (Zar 1999), as the assumption of homogeneity was not met. Statistical analyses were performed with SPSS ver 21.0.

The level of multi- paternity and maternity was used to assess the genetic mating system of the two species. Therefore, based on parentage analysis it was assessed whether males mated with one or more females and whether the same mother sired juveniles in one or more pregnant males.

5.2.6. Morphometric analysis

5.2.6.1. Landmark-based morphometric protocol

Digital images of the 1st day juveniles of *S. abaster* and *S. typhle* species, in a standard position pointing to the left, were analyzed. The landmarks that were selected were the same as the ones used in adults' morphometric analysis in Chapter 4 (Figure 5.5.).

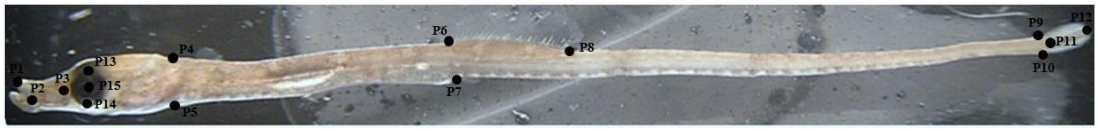


Figure 5.5. Landmarks used in the morphometric analysis of 1st day juveniles of *S. abaster* and *S. typhle* species born under reared conditions in the present study, shown on a figure of *S. abaster* juvenile.

Εικόνα 5.5. Ορόσημα που χρησιμοποιήθηκαν για τη μορφομετρική ανάλυση των νεαρών ατόμων (1ης ημέρας) των ειδών *S. abaster* και *S. typhle*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες κατά τη διάρκεια της παρούσας μελέτης. Η απεικόνιση πραγματοποιείται σε νεαρό άτομο του είδους *S. abaster*.

5.2.6.2. Statistical analysis

The procedure of the statistical analysis is described in detail in Chapter 4. Briefly, body size was estimated as centroid size. The raw coordinates of all specimens were aligned using the Procrustes generalized orthogonal least-squares (GLS) superimposition method. Principal Components Analysis (PCA) of the aligned coordinates was used to reduce the number of dimensions to the actual shape space dimensionality. The PC scores were used as variables in the Linear Discriminant Analysis (LDA) (Clunies-Ross and Riffenburgh 1960). The discriminatory effectiveness was determined from the percentage of correct classifications in a leave-one-out cross validation for linear discriminant analysis (Venables and Ripley 2002). All morphometric and statistical analyses were performed with MorphoJ, R-project MASS package (Venables and Ripley 2002) and SPSS ver. 21.0.

5.3. RESULTS

5.3.1. *Syngnathus abaster* species

Eleven (11) pregnant males of *S. abaster* species gave birth under laboratory conditions in the present study. The males exhibited a mean total length of 108.6 mm (ranging from 87 to 120 mm), a mean total weight of 0.520 gr (ranging from 0.262 to 0.616 gr) and a mean number of 27 dorsal fin rays (ranging from 26 to 29) (Table 5.3).

5.3.1.1. Notes on male pregnancy and parturition of *Syngnathus abaster* species

Pregnant males spent most of their time swimming near the bottom of the aquarium or lying in the lower parts of the artificial vegetation. In the early stages of pregnancy males were mobile, especially during feeding time. Their pouch was stiff and its color resembled that of the rest of the body (Figure 5.6a). As the pregnancy progressed, males spent most of their time lying in the bottom or resting in the artificial vegetation showing reduced mobility during feeding time. The texture of the pouch softened, its colored turned to grey-black and grew in size (Figure 5.6b, c). During that period the embryos had hatched from their eggs and were wrapped in the pouch (Figure 5. 6b, c). When, the embryonic development was completed, fully formed juveniles were ready to be released (Figure 5.6c).

Figure 5.6. Visible changes in the complexion and the texture of the brood pouch as recorded in *S. abaster* pregnant males of the present study, a) the pouch is stiff and the inseminated eggs are visible, b) eye-pigmentation of the embryos, c) the marsipium softens, grows in size as embryos hatched from their eggs and have developed into fully formed juveniles ready to be released.
Εικόνα 5.6. Αλλαγές στην υφή και στην εμφάνιση του εμβρυικού σάκου των αρσενικών κυοφορούντων ατόμων της παρούσας μελέτης, a) ο σάκος είναι σκληρός και τα γονιμοποιημένα αυγά είναι εμφανή, b) εμφανώς σχηματισμένος και χρωματισμένος οφθαλμός, c) ο σάκος είναι μαλακός, έχει μεγαλώσει σε μέγεθος ενώ τα έμβρυα έχουν εκκολαφθεί από τα αβγά τους, έχουν αναπτυχθεί και είναι έτοιμα να εκκολαφθούν.

Table 5.3. Descriptive statistics for the total length (TL, mm), total weight (TW, mm) and the number of dorsal fin rays (NDFR) of the eleven pregnant males of *S. abaster* that gave birth under reared conditions in the present study.
Πίνακας 5.3. Περιγραφική στατιστική ανάλυση του ολικού μήκος (TL, mm), ολικού βάρους (TW, gr) και του αριθμού των ακτίνων του ραχιαίου πτερυγίου (NDFR) των κυοφορούντων αρσενικών των ειδών *S. abaster* τα οποία γέννησαν σε εργαστηριακές συνθήκες κατά τη διάρκεια της παρούσας μελέτης.

Date of birth	Coloration	TL	TW	NDFR	Mean TL	Mean TW	Mean NDFR
7/5/2012	light green	107	0.522	28			
14/5/2012	brown-green	106	0.588	28			
29/5/2012	light green	117	0.577	28			
2/6/2012	light brown-green	120	0.616	26			
2/6/2012	dark green brown	87	0.262	27			
7/6/2012	green	116	0.526	27	108.6	0.520	27
8/6/2012	dark brown green	104	0.52	29			
3/7/2012	green	105	0.415	27			
9/7/2012	brown green	113	0.562	28			
13/7/2012	green	111	0.598	27			
18/7/2012	green	109	0.565	27			

On the day of the parturition the animal could barely move and the pouch was almost ready to open. Most of the males (82 %) gave birth during the night or in the early morning (between 9:00 pm and 9 am). In the beginning of the parturition, males started rolling and twisting their body. Even though *S. abaster* is a benthic species, during these movements they were not swimming in the bottom of the aquarium but acquired a more upright position almost vertical to the substratum. Fully formed juveniles, resembling the adults, were released from the marsupium by sharp bending movements of the male's body. The brood pouch opened either in the upper end, in the middle or in the lower end. The pseudo-placenta and the embryos were released as the fissure moved like a "zipper" downwards, in both directions or upwards respectively. In all studied males only one expanding fissure was noted - and never two or more - from which juveniles could be released.

Juveniles were released in batches. Before each release the male twisted and bended his body indicating a stressful state of labor and contractions. In the beginning few individuals were born sporadically, with intervals up to an hour between each release. The majority of the offspring were released in one big or two smaller batches. The remaining juveniles were released in sporadic batches, similar to the beginning of the labor. The juveniles could be released with the tail or the head following no particular pattern (Figure 5.7). During the intervals of successive batches, the pregnant male would stay still - probably resting- in the bottom or in the artificial plant exhibiting reduced or absent signs

of movement. At the end of the parturition along with the last newborns the pseudo-placenta tissue was also detached. Newborn juveniles of *S. abaster* spent most of the time near the bottom hiding or swimming in the sand, with only some sporadic movements towards the surface, followed by a return to the bottom section of the aquaria (Figure 5.8).

Even though adult males were regularly fed during the parturition and right after, filial cannibalism was recorded almost in all males.

Figure 5.7. Newborn specimen of *S. abaster* species being released from the brood pouch of a pregnant male during the present study.
Εικόνα 5.7. Νεογέννητο άτομο του είδους *S. abaster* καθώς ελευθερώνεται από τον εμβρυικό σάκο του κυοφορούντος αρσενικού, κατά τη παρούσα μελέτη.

Figure 5.8. Vertical distribution (near the bottom of the aquarium) of *S. abaster* 1st day juveniles, acquired right after abandoning the marsupium. The juveniles were born under reared conditions in the present study.
Εικόνα 5.8. Κατανομή στη στήλη του νερού του ενυδρείου των νεαρών ατόμων (1ης ημέρας) του είδους *S. abaster* (κοντά στον πυθμένα), τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες κατά τη διάρκεια της παρούσας μελέτης

5.3.1.2. 1st day juveniles of *Syngnathus abaster* species

The eleven pregnant males that gave birth under laboratory conditions released 473 juveniles (average number of 43 juveniles per male) . The total length, total weight and number of dorsal fin rays of the 1st day juveniles ranged from 13.0 to 22.0 mm (Mean TL= 17.mm), 0.001 to 0.005 gr (mean TW= 0.003 gr) and 24 to 30 (mean NDFR= 28) respectively (Table 5.4). The above mentioned measurements were not correlated with the number of released newborns (Spearman's correlation coefficient: r=0.528, p>0.05 and r=0.487, p>0.05 respectively).

Table 5.4. Total length (TL, mm), total weight (TW, mm) and number of dorsal fin rays (NDFR) of *S. abaster* 1st day juvenile specimens born under reared conditions in the present study (*N*, number of individuals; *Mean*, mean value*; Min*, minimum value; *Max*, maximum value*; St.Dev*, standard deviation).

Πίνακας 5.4. Ολικό μήκος (TL, mm), ολικό βάρος (TW, gr) και αριθμός ακτίνων του ραχιαίου πτερυγίου (NDFR) των νεαρών ατόμων (1ης ημέρας) του είδους *S. abaster*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες κατά τη διάρκεια της παρούσας μελέτης (*N*, αριθμός ατόμων; *Mean*, μέση τιμή*; Min*, ελάχιστη τιμή; *Max*, μέγιστη τιμή*; St.Dev*, τυπική απόκλιση).

Species		Mean	Min	Max	St.Dev
S. abaster (N = 473)	TL	17.7	13.0	22.0	0.18
	TW	0.003	0.001	0.005	0.0008
	NDFR	28	24	30	1.04

Even though the total length, total weight and number of dorsal fin rays did not vary statistically between pregnant males, differences were recorded between their offspring (Table 5.5). More specifically, juveniles born in 7/6/2012 and 8/6/2012 were the shortest and lightest, while those born in 29/5/2012 the longest and heaviest. Three intermediate length and two weight groups were recorded (Figure 5.9). Total length and total weight covaried (Spearman's correlation coefficient: r=0.874, p<0.001). Larger individuals were the heaviest and vice versa. The smallest number of dorsal fin rays were recorded in individuals born in 3/7/2012 (mean number of 26) while the most in 8/6/2012 and 9/7/2012 (mean number of 28 rays respectively). The individuals from the rest of the fathers formed one large intermediate group (Figure 5.9).

Table 5.5. Total length (TL, mm), total weight (TW, mm) and the number of dorsal fin rays (NDFR) among *S. abaster* 1st day juvenile specimens born from eleven different pregnant males under reared conditions in the present study (*N*, number of individuals; *Mean*, mean value*; Min*, minimum value; *Max*, maximum value*; St.Dev*, standard deviation).

Πίνακας 5.5. Ολικό μήκος (TL, mm), ολικό βάρος (TW, gr) και αριθμός ακτίνων του ραχιαίου πτερυγίου (NDFR) μεταξύ νεαρών ατόμων (1ης ημέρας) του είδους *S. abaster*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες από έντεκα διαφορετικούς αρσενικούς γεννήτορες κατά τη διάρκεια της παρούσας μελέτης (*N*, αριθμός ατόμων; *Mean,* μέση τιμή*; Min*, ελάχιστη τιμή; *Max*, μέγιστη τιμή*; St.Dev*, τυπική απόκλιση).

Date of birth		Mean	Min	Max	St.Dev
7/5/2012 (N=27)	TL	17.8	15.0	20.0	0.14
	TW				
	NDFR	28	27	29	0.42
14/5/2012 (N=49)	TL	18.3	17.0	20.0	0.10
	TW	0.003	0.002	0.005	0.0006
	NDFR	28	26	30	1.04
29/5/2012 (N=63)	TL	19.7	17.0	22.0	0.14
	TW	0.004	0.002	0.005	0.0009
	NDFR	28	26	30	0.69
2/6/2012 (N=67)	TL	18.2	14.0	20.0	0.17
	TW	0.003	0.001	0.005	0.0009
	NDFR	27	25	29	0.80
2/6/2012 (N=33)	TL	18.2	16.0	19.0	0.07
	TW	0.003	0.003	0.004	0.0005
	NDFR	28	27	29	0.65
7/6/2012 (N=48)	TL	15.3	13.0	18.0	0.12
	TW	0.002	0.002	0.003	0.0005
	NDFR	28	26	30	0.79
8/6/2012 (N=42)	TL	15.0	13.0	16.0	0.09
	TW	0.003	0.001	0.003	0.0006
	NDFR	28	26	30	0.89
3/7/2012 (N=37)	TL	17.5	16.0	19.0	0.08
	TW	0.003	0.002	0.004	0.0005
	NDFR	26	25	28	0.70
9/7/2012 (N=39)	TL	17.8	16.0	19.0	0.08
	TW	0.003	0.002	0.004	0.0003
	NDFR	28	25	30	1.02
13/7/2012 (N=29)	TL	18.8	16.0	21.0	0.11
	TW	0.003	0.002	0.005	0.0007
	NDFR	28	26	30	1.10
18/7/2012 (N=39)	TL	17.3	15.0	19.0	0.11
	TW	0.003	0.002	0.004	0.0006
	NDFR	27	24	29	1.24

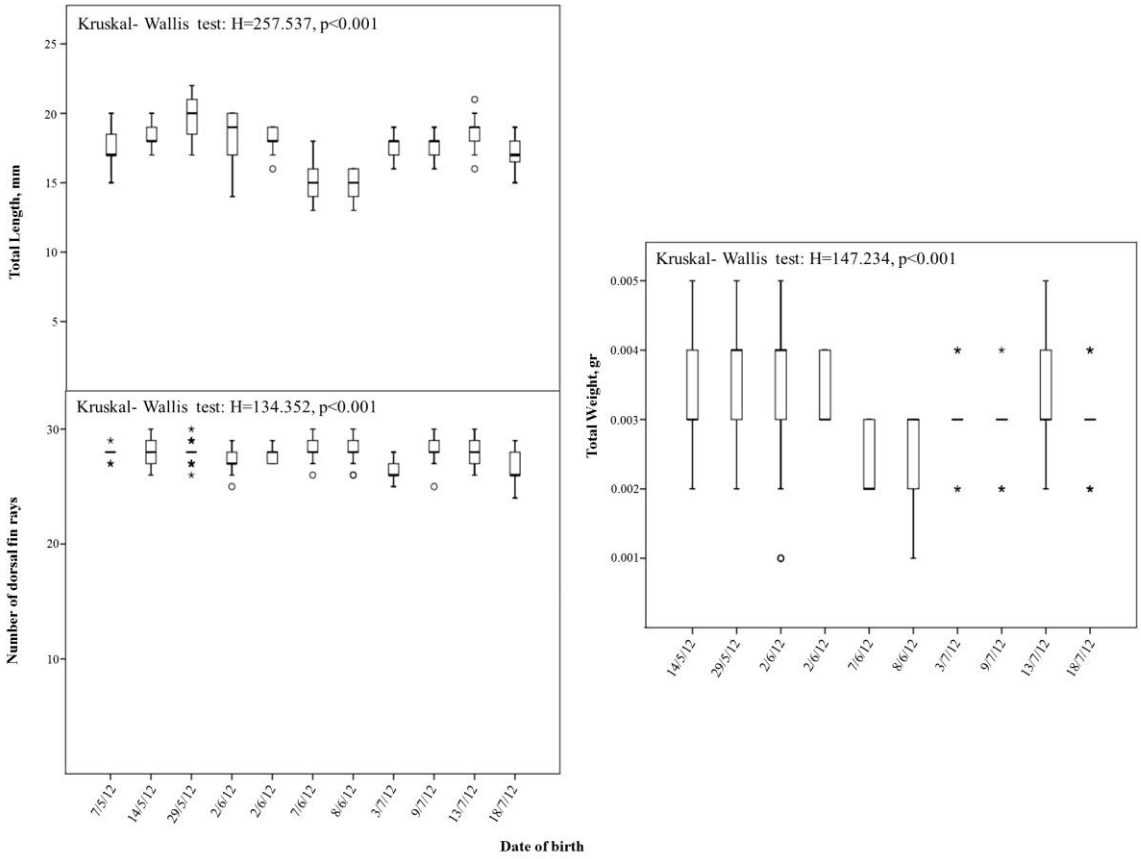


Figure 5.9. Box plots of the total length (TL, mm), total weight (TW, mm) and the number of dorsal fin rays (NDFR) among *S. abaster* 1st day juvenile specimens born from eleven different pregnant males under reared conditions in the present study (*H*, Value of Kruskal-Wallis non parametric test; *P*, level of significance, the middle bold black line within each box corresponds to the median, the points are outliers, the asterisks are extreme outliers and the T-bars that extend from the boxes correspond to the standard deviation).

Εικόνα 5.9. Θηκογράμματα του ολικού μήκος (TL, mm), ολικού βάρους (TW, gr) και του αριθμού των ακτίνων του ραχιαίου πτερυγίου (NDFR) μεταξύ νεαρών ατόμων (1ης ημέρας) του είδους *S. abaster*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες από έντεκα διαφορετικούς αρσενικούς γεννήτορες κατά τη διάρκεια της παρούσας μελέτης (*H*, η τιμή του μη- παραμετρικού τεστ Kruskal-Wallis; *P*, επίπεδο σημαντικότητας, η μεσαία έντονη γραμμή κάθε θηκογράμματος αντιστοιχεί στο διάμεσο, οι κύκλοι σε ακραίες τιμές, οι αστερίσκοι σε εξαιρετικά ακραίες τιμές και οι γραμμές σχήματος «Τ» στην τυπική απόκλιση του δείγματος).

In the day of parturition, within each birth, most offspring were of the same length and weight and had the same number of dorsal fin rays (Figure 5.10), while their coloration varied from a light shade (light green or brown), to a darker (dark green or brown) or even black (Figure 5.11, Table 5.6). No important statistical differences were recorded in the total length, total weight and number of dorsal fin rays of different colored juveniles (Table 5.7). Exceptions to this pattern were the births on the 7/6/2012 and 18/7/2012, when juveniles with black or dark brown body colors were statistically shorter than the rest of their siblings (Figure 5.12). Additionally, dark colored specimens born on 2/6/2012 and black colored born on 7/6/2012 had statistically more and less dorsal fin rays respectively, than their siblings (Figure 5.12).

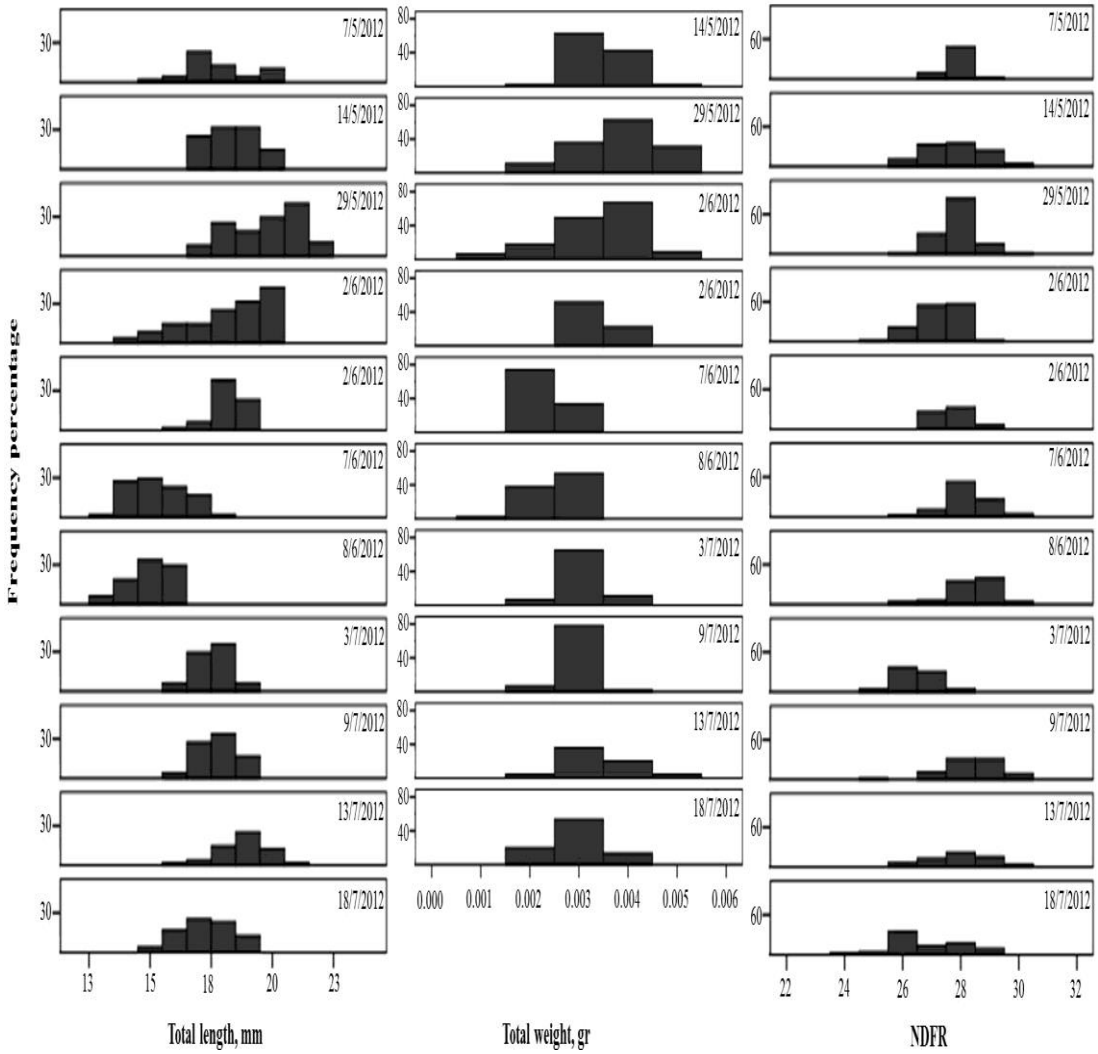


Figure 5.10. Total length, total weight and number of dorsal fin rays (NDFR) distribution of 1st day offspring of *S. abaster* species born under reared conditions in the present study.

Εικόνα 5.10. Κατανομή ολικού μήκους, ολικού βάρους και αριθμού των ακτίνων του ραχιαίου πτερυγίου των νεαρών απογόνων (1ης ημέρας) του είδους *S. abaster*, τα οποία γεννήθηκαν υπό εργαστηριακές συνθήκες κατά την παρούσα μελέτη.

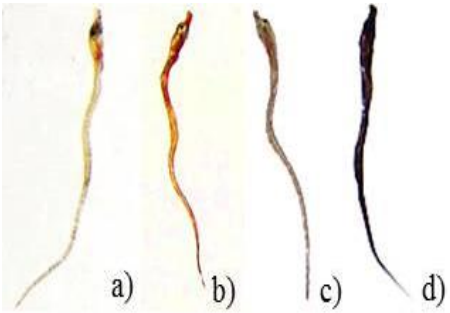


Figure 5.11. Color paterns of *S. abaster* 1st day juvenile specimens born from pregnant males under reared conditions in the present study. *a*, light brown; *b*, brown; *c*, dark brown; *d*, black.

Εικόνα 5.11. Χρωματικά πρότυπα των νεαρών ατόμων (1ης ημέρας) του είδους *S. abaster*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες από αρσενικούς γεννήτορες κατά τη διάρκεια της παρούσας μελέτης. *α,* ανοιχτό καφέ; *b*, καφέ; *c*, σκούρο καφέ; *d*, μαύρο .

T**able 5.6.** Total length (TL, mm), total weight (TW, mm) and number of dorsal fin rays (NDFR) according to the color of the 1st day juveniles of *S. abaster* species, born from eleven different pregnant males under reared conditions in the present study (*N*, number of individuals; *Mean*, mean value*; Min*, minimum value; *Max*, maximum value*; St.Dev*, standard deviation).

Πίνακας 5.6. Ολικό μήκος (TL, mm), ολικό βάρος (TW, gr) και αριθμός ακτίνων του ραχιαίου πτερυγίου (NDFR) σε σχέση με το χρώμα των νεαρών ατόμων (1ης ημέρας) του είδους *S. abaster*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες από έντεκα διαφορετικούς αρσενικούς γεννήτορες κατά τη διάρκεια της παρούσας μελέτης (*N*, αριθμός ατόμων; *Mean*, μέση τιμή*; Min*, ελάχιστη τιμή; *Max*, μέγιστη τιμή*; St.Dev*, τυπική απόκλιση).

Date of birth	Color		Mean	Min	Max	St.dev
7/5/2012 (N=27)	(N=27)	TL	17.8	15.0	20.0	0.14
		TW				
		NDFR	28	27	29	0.42
14/5/2012 (N=49)	Light brown (N=48)	TL	18.4	17.0	20.0	0.10
		TW	0.003	0.002	0.005	0.0006
		NDFR	28	26	30	1.05
	Dark Brown (N=1)	TL	18.0	18.0	18.0	
		TW	0.004	0.004	0.004	
		NDFR	28	28	28	
29/5/2012 (N=63)	Light brown (N=26)	TL	19.7	17.0	22.0	0.17
		TW	0.004	0.002	0.005	0.0009
		NDFR	28	26	29	0.63
	Dark brown (N=37)	TL	19.8	18.0	22.0	0.12
		TW	0.004	0.002	0.005	0.0009
		NDFR	28	27	30	0.70
2/6/2012 (N= 67)	Light brown (N=29)	TL	18.7	16.0	20.0	0.16
		TW	0.004	0.001	0.005	0.0009
		NDFR	27	25	29	0.89
	Brown (N=21)	TL	18.0	14.0	20.0	0.19
		TW	0.003	0.001	0.004	0.0010
		NDFR	27	26	28	0.71
	Dark brown (N=17)	TL	17.7	14.0	19.0	0.15
		TW	0.003	0.001	0.005	0.0008
		NDFR	28	27	28	0.39
2/6/2012 (N=33)	Light green (N=33)	TL	18.2	16.0	19.0	0.07
		TW	0.003	0.003	0.004	0.0005
		NDFR	28	27	29	0.65

7/6/2012 (N=48)	Light green (N=14)	TL	15.4	13.0	17.0	0.12
		TW	0.002	0.002	0.003	0.0005
		NDFR	28	28	30	0.63
	Dark green (N=26)	TL	15.6	14.0	18.0	0.12
		TW	0.002	0.002	0.003	0.0005
		NDFR	28	27	30	0.78
	Black (N=8)	TL	14.3	14.0	15.0	0.05
		TW	0.002	0.002	0.003	0.0005
		NDFR	27	26	28	0.79
8/6/2012 (N=42)	Light green (N=22)	TL	15.1	13.0	16.0	0.10
		TW	0.003	0.001	0.003	0.0006
		NDFR	28	26	30	1.05
	Dark green (N=20)	TL	14.9	13.0	16.0	0.09
		TW	0.003	0.002	0.003	0.0005
		NDFR	28	27	29	0.67
3/7/2012 (N=37)	Light brown (N=14)	TL	17.4	16.0	19.0	0.08
		TW	0.003	0.002	0.004	0.0007
		NDFR	26	25	28	0.76
	Brown (N=22)	TL	17.6	16.0	19.0	0.08
		TW	0.003	0.003	0.004	0.0003
		NDFR	27	25	28	0.69
	Black (N=1)	TL	17.0	17.0	17.0	
		TW	0.003	0.003	0.003	
		NDFR	26	26	26	
9/7/2012 (N=39)	Light brown (N=13)	TL	18.0	16.0	19.0	0.08
		TW	0.003	0.002	0.004	0.0006
		NDFR	29	27	30	0.93
	Brown (N=19)	TL	17.7	16.0	19.0	0.09
		TW	0.003	0.003	0.003	0.0000
		NDFR	28	27	30	0.77
	Dark brown (N=7)	TL	17.4	17.0	19.0	0.08
		TW	0.003	0.003	0.003	0.0000
		NDFR	28	25	29	1.50
13/7/2012 (N=29)	Light brown(N=16)	TL	18.8	17.0	21.0	0.10
		TW	0.003	0.003	0.004	0.0005
		NDFR	28	26	30	1.29
	brown(N=10)	TL	19.0	17.0	20.0	0.09
		TW	0.004	0.002	0.005	0.0010
		NDFR	28	26	29	0.88
	Dark brown (N=3)	TL	18.0	16.0	20.0	0.20
		TW	0.003	0.002	0.003	0.0006
		NDFR	28	27	28	0.71
18/7/2012 (N=39)	Brown (N=23)	TL	17.6	16.0	19.0	0.11
		TW	0.003	0.002	0.004	0.0005
		NDFR	27	24	29	1.30
	Dark brown (N=16)	TL	16.8	15.0	19.0	0.10
		TW	0.003	0.002	0.004	0.0008
		NDFR	27	25	29	1.20
3/6/2012 (N=78)	Light green (N=46)	TL	25.7	24.0	28.0	0.10
		TW	0.006	0.004	0.009	0.0011
		NDFR	33	30	35	1.09
	Dark green (N=32)	TL	26.5	21.0	29.0	0.17
		TW	0.007	0.003	0.009	0.0015
		NDFR	33	30	35	1.41

Table 5.7. Results of the non-parametric Kruskal- Wallis test (H) for the total length (TL, mm), total weight (TW, mm) and the number of dorsal fin rays (NDFR) according to the color of the 1st day juveniles of *S. abaster* species, born from eleven different pregnant males under reared conditions in the present study (*N*, number of individuals; *P-values*, level of significance).
Πίνακας 5.7. Αποτελέσματα του μη-παραμετρικού ελέγχου κατά Kruskal-Wallis (H) για το ολικό μήκος (TL, mm), ολικό βάρος (TW, gr) και αριθμό ακτίνων του ραχιαίου πτερυγίου (NDFR) σε σχέση με το χρώμα των νεαρών ατόμων (1ης ημέρας) του είδους *S. abaster*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες από έντεκα διαφορετικούς αρσενικούς γεννήτορες κατά τη διάρκεια της παρούσας μελέτης (*N*, αριθμός ατόμων; *P-values*, επίπεδο σημαντικότητας).

Birth		H	P-values
14/5/2012 (N=49)	TL	0.135	>0.05
	TW	1.297	>0.05
	NDFR	0.066	>0.05
29/5/2012 (N=63)	TL	0.004	>0.05
	TW	0.37	>0.05
	NDFR	3.214	>0.05
2/6/2012 (N=67)	TL	5.323	>0.05
	TW	3.914	>0.05
	NDFR	**14.223**	**<0.001**
7/6/2012 (N=48)	**TL**	**9.036**	**<0.05**
	TW	0.191	>0.05
	NDFR	**7.024**	**<0.05**
8/6/2012 (N=42)	TL	0.841	>0.05
	TW	0.017	>0.05
	NDFR	0.519	>0.05
3/7/2012 (N=37)	TL	1.656	>0.05
	TW	0.268	>0.05
	NDFR	0.709	>0.05
9/7/2012 (N=39)	TL	2.998	>0.05
	TW	2.167	>0.05
	NDFR		
13/7/2012 (N=29)	TL	1.238	>0.05
	TW	3.243	>0.05
	NDFR	3.321	>0.05
18/7/2012 (N=39)	**TL**	**4.638**	**<0.05**
	TW	0.946	>0.05
	NDFR	0.803	>0.05

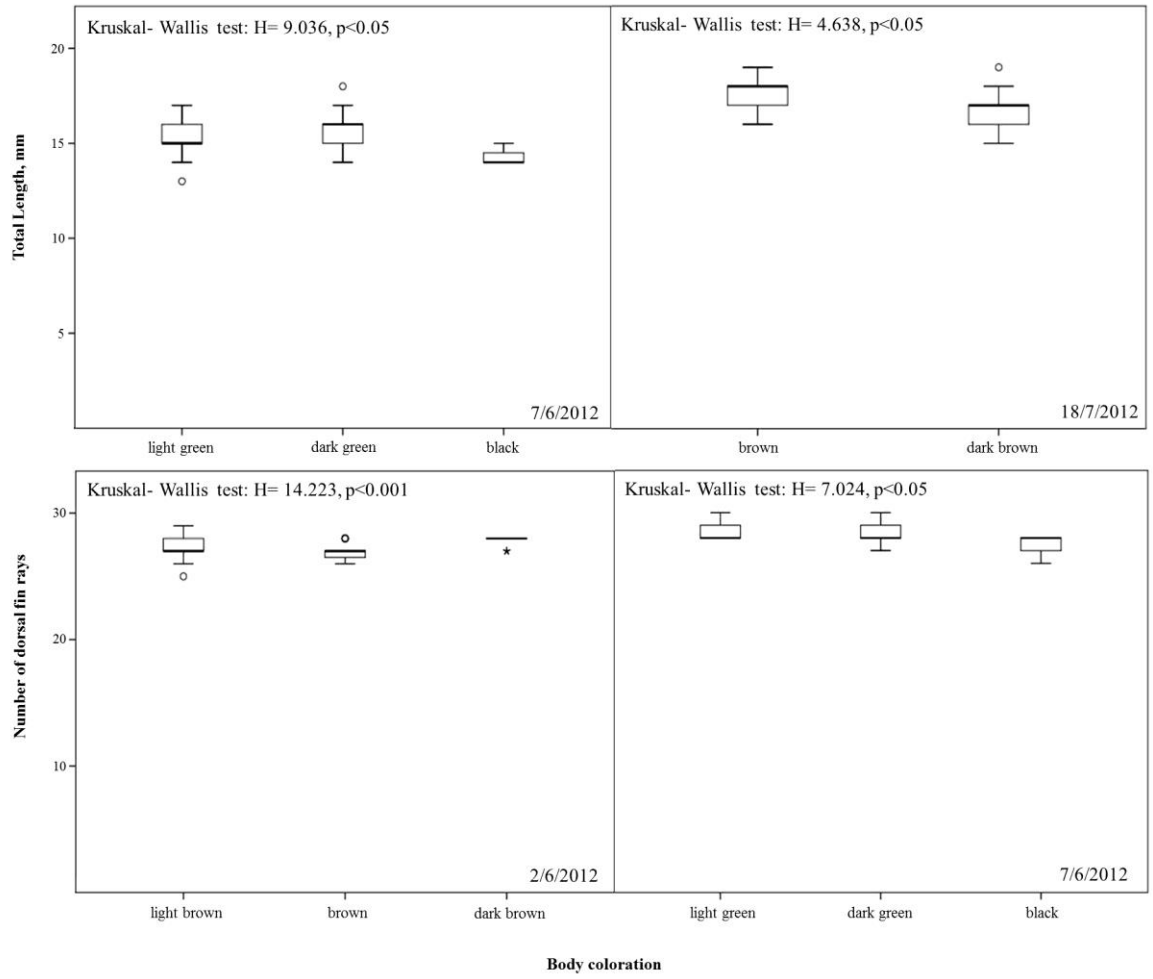


Figure 5.12. Box plots of the statistically important differences in the total length (TL, mm) and number of dorsal fin rays (NDFR) according to the color of the 1st day juveniles of *S. abaster* species, born under reared conditions in the present study (*H*, Value of Kruskal-Wallis non parametric test; *P*, level of significance, the middle bold black line within each box corresponds to the median, the points are outliers, the asterisks are extreme outliers and the T-bars that extend from the boxes correspond to the standard deviation).

Εικόνα 5.12. Θηκογράμματα των στατιστικά σημαντικών διαφορών του ολικού μήκος (TL, mm) και του αριθμού των ακτίνων του ραχιαίου πτερυγίου (NDFR) σε σχέση με το χρώμα των νεαρών ατόμων (1ης ημέρας) του είδους *S. abaster*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες κατά τη διάρκεια της παρούσας μελέτης (*H*, η τιμή του μη- παραμετρικού τεστ Kruskal-Wallis; *P*, επίπεδο σημαντικότητας, η μεσαία έντονη γραμμή κάθε θηκογράμματος αντιστοιχεί στο διάμεσο, οι κύκλοι σε ακραίες τιμές, οι αστερίσκοι σε εξαιρετικά ακραίες τιμές και οι γραμμές σχήματος «Τ» στην τυπική απόκλιση του δείγματος).

5.3.1.3. Parentage analysis and genetic mating system of *Syngnathus abaster* species

The selected pregnant males (N=8), a subset of their offspring (N=249) and thirty four wild individuals of Drepano population were successfully genotyped at each of the four loci (Table 5.1 in Appendix). All loci were variable as the number of alleles per locus varied from eighteen at S.abas3 (14 in WP) to forty four at S.abas9 (27 in WP) (Table 5.8). The average expected heterozygosity over loci was 0.95 (ranging from 0.92 at S.abas3 to 0.96 at S.abas7 and 9) and the mean polymorphic information content (PIC) value was 0.93 (ranging from 0.90 at S.abas3 to 0.94 at S.abas7 and 9) (Table 5.8). The high values of both indices revealed that these markers are highly informative for paternity estimation (Botstein et al. 1980). Significant deviation from the Hardy-Weinberg equilibrium at a 0.05 α-level was not detected in any loci (Table 5.8), indicating that the presence of null alleles was far too rare in the population to complicate mating system analysis.

Table 5.8. Genetic variability of *S. abaster* species in the population of Drepano at 4 microsatellite loci used for parentage analysis as revealed by the allele frequency, heterozygosity values, Hardy-Weinberg test and Polymorphic Information Content for each locus of the wild population genotyped in the present study (*NA*, number of alleles found in offspring and wild population; *, alleles found in offspring; F_A, allele frequency in the wild population; *k* number of alleles found in the wild population; *N*, number of individuals genotyped per locus; H_o, observed heterozygosity; H_e, expected heterozygosity; *PIC*, polymorphic information content; *HW*, values of Hardy-Weinberg test; F_N, null allele frequency; *NS*, non-significant).

Πίνακας 5.8. Γενετική ποικιλομορφία του είδους *S. abaster* στον πληθυσμό του Δρεπάνου στους 4 μικροδορυφορικούς τόπους που χρησιμοποιήθηκαν στον έλεγχο της μητρότητας. Το επίπεδο της ποικιλομορφίας προκύπτει από την συχνότητα των αλληλομόρφων, τον έλεγχο της ισορροπίας Hardy-Weinberg, το δείκτη πολυμορφισμού των μικροδορυφορικών τόπων και ετεροζυγωτίας σε κάθε τόπο του άγριου πληθυσμού του είδους στη παρούσα μελέτη (*NA*, ο αριθμός των αλληλομόρφων του άγριου πληθυσμού και των απογόνων, *, αλληλόμορφα απογόνων; F_A, συχνότητα των αλληλομόρφων του άγριου πληθυσμού; *k*, ο αριθμός των αλληλομόρφων του άγριου πληθυσμού; *N*, αριθμός ατόμων που γενοτυπήθηκε ανά τόπο; H_o, παρατηρούμενη ετεροζυγωτία; H_e, αναμενόμενη ετεροζυγωτία; *PIC*, δείκτη πολυμορφισμού των μικροδορυφορικών τόπων; *HW*, τιμές του ελέγχου ισορροπίας Hardy-Weinberg; F_N, συχνότητα μηδενικών αλληλομόρφων; *NS*, μη στατιστικά σημαντικό).

			Wild population							
Locus	**NA**	**Allele**	**F_A**	**K**	**N**	**H_o**	**H_e**	**PIC**	**HW**	**F_N**
S.abas3	18	166*		14	25	1.00	0.92	0.90	NS	-0.05
		174*								
		178*	0.04							
		182*	0.04							
		186*	0.06							
		190*	0.10							
		194*	0.12							
		198*	0.12							
		202*	0.16							
		206*	0.10							
		210*	0.08							
		214*								
		218*	0.08							
		222*	0.04							
		226	0.02							
		234*	0.02							
		238*								
		242*	0.02							

		193*								
		197*								
		209*								
		217	0.02							
		221*	0.03							
		225*	0.05							
		229*	0.05							
		237*	0.06							
		241*	0.06							
		245*	0.09							
		249*	0.05							
		253*	0.09							
		257*	0.12							
		261*	0.05							
S.abas4	29	265*	0.06	23	33	0.97	0.95	0.93	NS	-0.02
		269*	0.08							
		273*	0.02							
		277*	0.02							
		281*	0.02							
		285*	0.02							
		289*								
		293*	0.03							
		297*	0.03							
		301	0.02							
		305*	0.05							
		309	0.02							
		317	0.02							
		345*								
		361*								
		206*	0.01							
		210	0.01							
		214*								
		218*	0.01							
		222	0.01							
		226*	0.04							
		230*	0.09							
		234*	0.06							
		238*	0.04							
		242*	0.03							
		246*	0.06							
		250*	0.06							
		254*	0.07							
S.abas7	25	258*	0.12	24	34	0.97	0.96	0.94	NS	-0.01
		262*	0.06							
		266*	0.03							
		270*	0.06							
		274*	0.04							
		278*	0.04							
		282*								
		286*	0.03							
		290*	0.03							
		294*	0.01							
		298*	0.03							
		302*	0.01							
		346	0.01							
		192*								
		200*								
		204*								
S.abas9	34	208*		27	26	0.96	0.96	0.94	NS	-0.01
		212*	0.02							
		216*	0.04							

	220*	0.02
	224	0.04
	228*	0.02
	232*	0.02
	236*	0.02
	240*	0.02
	252	0.02
	264	0.02
	268	0.02
	336	0.02
	340*	0.02
	352	0.02
	360*	
	364	0.04
	372*	
	376*	0.06
	380*	0.06
	384*	0.08
	388*	0.12
	392*	0.10
	396*	0.08
	400*	0.04
	404*	0.04
	408*	0.02
	412	0.04
	416*	0.02
	420*	0.02
	424*	

Colony software assigned all embryos to their known father without mismatches. Thus, there is complete confidence of paternity of the broods for each *S. abaster* male. In total, according to the reconstruction of maternal genotypes at least twenty eight females contributed to the broods of pregnant males (Table 5.2 in Appendix), none of which was caught during sampling. As males mated in the wild and the genotype of the candidate mothers was reconstructed the software cannot distinguish whether offspring were sired by one mother or multiple with the same combination of alleles. Given that in each locus the levels of PIC and expected heterozygosity were high, the possibility of two or more individuals sharing the exact same combination of alleles in all four loci is very small, though not impossible. Therefore, this is the minimum number of expected mothers. The high possibility of i) correct reconstruction of the maternal genotype (75%) (Table 5.2 in Appendix) and ii) assignment of offspring genotype to these mothers (94%), indicate that the minimum number of potential mothers was probably correct.

Each male received eggs from more than one female. The mean number of successful mates per male was 4.25 (St.Dev=1.39) ranging from 3 to 6 mates (Table 5.9). Within each brood, the number of offspring sired by each mother varied from one (mothers ID: #8, #3, #20, #21, 13#) to twenty four (mother ID: #1) (Table 5.9). Besides multiple mating, the occurrence of different males' offspring with identical mother genotypes (mothers ID: #3, #13, #16, #17, #21) was observed (Table 5.9). Mothers with ID #3, #16, #17 and #21 sired juveniles in two broods while mother #13 sired embryos in three broods (Table 5.3).

Table 5.9. Description of the mating system of the eight pregnant males of *S. abaster* species that gave birth under laboratory conditions and were used for parentage analysis in the present study. The number of possible female donors and the number of their sired juveniles was estimated by the Colony software.
Πίνακας 5.9. Περιγραφή του αναπαραγωγικού συστήματος των οχτώ κυοφορούντων αρσενικών ατόμων του είδους *S. abaster*, τα οποία γέννησαν σε εργαστηριακές συνθήκες και χρησιμοποιήθηκαν στην ανάλυση μητρότητας. Ο αριθμός των πιθανών θηλυκών γεννητόρων και των απογόνων τους υπολογίστηκε από το πρόγραμμα Colony.

Date of birth	Father ID	Number of juveniles	Genotyped juveniles	Possible mothers	Mother ID	Number of sired juveniles	Shared mothers	Shared mother ID
14/5/2012	G14	49	33	5	#4 #5 #6 #7 #8	9 10 10 3 1	0	
29/5/2012	G15	63	32	3	#1 #2 #3	24 7 1	1	 #3
2/6/2012	G16	67	31	6	#10 #11 #12 #13 #14 #9	6 6 5 2 3 9	1	 #13
7/6/2012	G8	48	33	3	#15 #16 #17	11 18 4	1	 #16 #17
8/6/2012	G9	42	31	5	#16 #17 #18 #19 #20	9 6 13 2 1	2	#16 #17
3/7/2012	G2	37	31	3	#21 #22 #23	4 19 8	1	#21
9/7/2012	G4	39	31	6	#13 #21 #24 #25 #26 #3	2 1 7 10 10 1	3	#13 #21 #3
13/7/2012	G10	29	27	3	#13 #27 #28	1 13 13	1	#13

Among the sired juveniles statistically important differences were recorded in their total length (Kruskal- Wallis test: H= 132.584, p<0.001) and the number of dorsal fin rays (Kruskal- Wallis test: H= 78.425, p<0.001). More specifically, concerning the total length, five subgroups were found. Offspring sired from mother #15 were the shortest while those from mothers #11 and #28 were the longest. The rest of the mothers sired intermediate sized offspring (Figure 5.13). Mother #22 sired juveniles with the least dorsal fin rays while mothers #20 and #26 with the most. Three intermediate subgroups were recorded (Figure 5.13.).

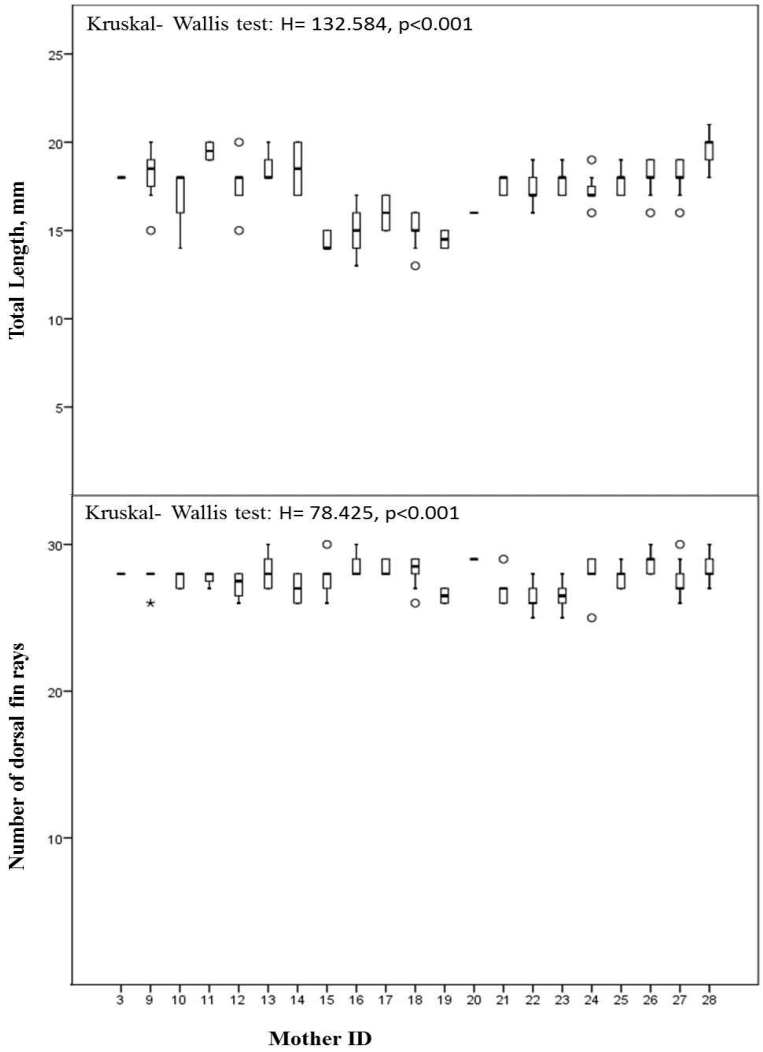


Figure 5.14. Boxplots of the total length (TL, mm) and the number of dorsal fin rays of the sired 1st day juveniles of *S. abaster* species from 28 mothers of in the present study (*H,* Value of Kruskal-Wallis non parametric test; *P*, level of significance, the middle bold black line within each box corresponds to the median, the points are outliers, the asterisks are extreme outliers and the T-bars that extend from the boxes correspond to the standard deviation).

Εικόνα 5.14. Θηκογράμματα του ολικού μήκους και του αριθμού των ραχιαίων ακτίνων των απογόνων των 28 θηλυκών γεννητόρων του είδους *S. abaster* κατά τη διάρκεια τη παρούσας μελέτης (*H*, η τιμή του μη- παραμετρικού τεστ Kruskal-Wallis; *P*, επίπεδο σημαντικότητας, η μεσαία έντονη γραμμή κάθε θηκογράμματος αντιστοιχεί στο διάμεσο, οι κύκλοι σε ακραίες τιμές, οι αστερίσκοι σε εξαιρετικά ακραίες τιμές και οι γραμμές σχήματος «Τ» στην τυπική απόκλιση του δείγματος).

5.3.2. *Syngnathus typhle* species

Three pregnant males of *S. typhle* species gave birth under reared conditions during the present study. They exhibited a mean total length of 169 mm (ranging from 150 to 181 mm), a mean total weight of 1.025 gr (ranging from 0.866 to 1.184 gr) and a mean number of 34 dorsal fin rays (ranging from 33 to 34) (Table 5.10).

5.3.2.1. Notes on male pregnancy and parturition of *Syngnathus typhle* species

Pregnant males spent most of their time swimming near the surface of the aquarium, staying in a vertical posture or lying in the upper parts of the artificial vegetation. Males' mobility and brood pouch complexion and texture followed the general pattern observed in *S. abaster* species. In particular, mobility of males decreased as pregnancy went along. At the same time, the brood pouch softened, got darker and bigger (Figure 5.14a-c). However, the marsupium of *S. typhle* males was less transparent compared to *S. abaster*. When the embryonic development was completed, fully formed juveniles were ready to be released.

Two of the pregnant males gave birth during the night or in the early morning and one during the day exhibiting a vertical posture. The labor was characterized by strong contractions as indicated by the sharp bents and twists of the body. The first juvenile was released from a fissure created either in the upper, middle or lower end of the marsupium. The fissure expanded in a "zipper" pattern downwards, in both directions or upwards respectively and similarly to *S. abaster* there was never more than one. As the fissure unraveled the pouch, the pseudo-placenta was also detached, protruding from the body (Figure 5.14d).

Fully formed juveniles, resembling adult individuals, were released from the marsupium sporadically. Between the releases, up to half hour intervals were recorded. Similarly to *S. abaster*, the majority of the offspring were released in one big or two smaller batches, leaving the pouch with the tail or the head following no particular pattern. During the intervals of successive batches, the pregnant male stayed still, probably resting. However, during feeding time it exhibited increased mobility, similar to the levels of early pregnancy stages. At the end of the parturition, along with the last juveniles the pseudo-placenta tissue was also fully detached from the body and discarded to the bottom of the aquarium.

Newborn juveniles of *S. typhle* species exhibited a vertical swim-up behavior near the surface of the aquarium (Figure 5.15). Their presence near the bottom was sparse and rare. Newborns were actively preyed by their own father –phenomenon of filial cannibalism- even though adult males were fed tactically.

Table 5.10. Total length (TL, mm), total weight (TW, mm) and number of dorsal fin rays (NDFR) of 1st day juvenile specimens of *S. typhle* species that gave birth under reared conditions in the present study.
Πίνακας 5.10. Ολικό μήκος (TL, mm), ολικό βάρος (TW, gr) και αριθμός ακτίνων του ραχιαίου πτερυγίου (NDFR) των κυοφορούντων αρσενικών του είδους *S. typhle*, τα οποία γέννησαν σε εργαστηριακές συνθήκες κατά τη διάρκεια της παρούσας μελέτης.

Parturition Date	Coloration	TL	TW	NDFR	Mean TL	Mean TW	Mean NDFR
6/5/2012	green	181					
11/5/2012	dark green	150	0.866	33			
3/6/2012	green	176	1.184	34	169	1.025	34

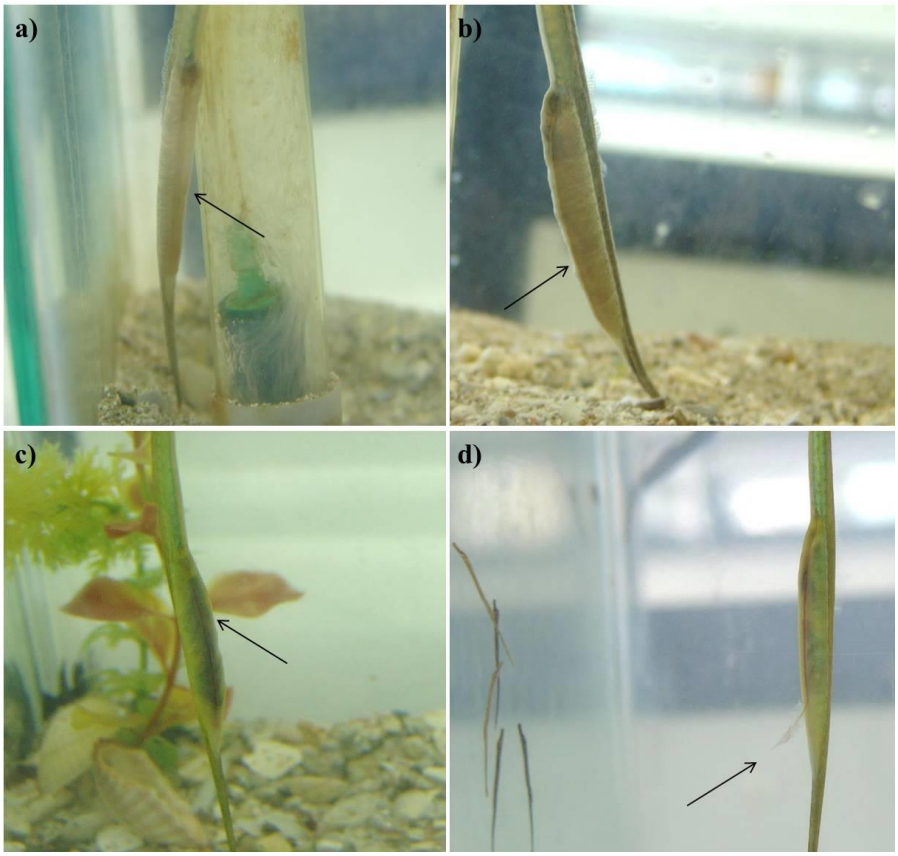


Figure 5.14 Visible changes in the complexion and the texture of the brood pouch as recorded in *S. typhle* pregnant males in the present study: a) the pouch is stiff and it color resembles the rest of the body, b) the pouch becomes less stiff and start to form a black line in the middle, c) the marsupium has a dark complexion, has grown in size and is soft, d) the first juveniles are released from a fissure in the lower end of the marsupium. The pseudo-placenta (depicted with the black arrow) detaches along with the embryos as the fissure moves upwards.
Εικόνα 5.14. Αλλαγές στην υφή και στην εμφάνιση του εμβρυικού σάκου αρσενικών κυοφορούντων ατόμων της παρούσας μελέτης: a) ο σάκος είναι σκληρός και το χρώμα του ίδιο με το υπόλοιπο σώμα, b) η υφή του σάκου γίνεται πιο μαλακή και ξεκινά να σχηματίζεται μαύρη γραμμή στη κεντρική περιοχή, c) ο σάκος είναι μαλακός και σκουρόχρωμος, ενώ έχει μεγαλώσει και σε μέγεθος, d) τα πρώτα νεαρά άτομα έχουν ελευθερωθεί από το άνοιγμα στο κάτω άκρο του μάρσιπου. Ο πλακούντας (σημαίνεται με μαύρο βέλος) αποκολλάται και προεξέχει από το μάρσιπο καθώς ελευθερώνονται οι απόγονοι.

Figure 5.15 Vertical distribution of *S. typhle* (near the surface of the aquarium) 1st day juveniles acquired right after abandoning the marsupium. A varying coloration pattern is obvious. The juveniles were born under reared conditions in the present study.
Εικόνα 5.15. Κατανομή στη στήλη του νερού του ενυδρείου των νεαρών ατόμων (1ης ημέρας) του είδους *S. typhle* (κοντά στην επιφάνεια του ενυδρείου). Οι απόγονοι γεννήθηκαν σε εργαστηριακές συνθήκες κατά τη διάρκεια της παρούσας μελέτης και είναι εμφανές το διαφορετικό χρωματικό πρότυπο.

5.3.2.2. 1st day juveniles of *Syngnathus typhle* species

During the present study, three pregnant males gave birth under laboratory conditions releasing 128 juveniles (average number of 43 juveniles per male). The total length and total weight of the males were not correlated with the number of released newborns (Spearman's rho test: r=0.500, p>0.05 and r=0.487, p>0.05 respectively). The total length, total weight and number of dorsal fin rays of the 1st day juveniles ranged from 21.0 to 30.0 mm (Mean TL= 26.2 mm), 0.003 to 0.010 gr (mean TW= 0.007 gr) and 30 to 35 (mean NDFR= 33) respectively (Table 5.11).

Table 5.11. Total length (TL, mm), total weight (TW, mm) and number of dorsal fin rays (NDFR) of *S. typhle* 1st day juvenile specimens born under reared conditions in the present study (*N*, number of individuals; *Mean*, mean value*; Min*, minimum value; *Max*, maximum value*; St.Dev*, standard deviation).
Πίνακας 5.11.Ολικό μήκος (TL, mm), ολικό βάρος (TW, gr) και αριθμός ακτίνων του ραχιαίου πτερυγίου (NDFR) των νεαρών ατόμων (1ης ημέρας) του είδους *S. typhle*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες κατά τη διάρκεια της παρούσας μελέτης (*N*, αριθμός ατόμων; *Mean*, μέση τιμή*; Min*, ελάχιστη τιμή; *Max*, μέγιστη τιμή*; St.Dev*, τυπική απόκλιση).

Species		Mean	Min	Max	St. Dev
S. typhle (N = 128)	TL	26.2	21.0	30.0	0.16
	TW	0.007	0.003	0.010	0.0016
	NDFR	33	30	35	1.07

Statistical important differences were recorded in the total length and the total weight among *S. typhle* 1st day juveniles (Figure 5.17, Table 5.12). In particular, juveniles born in 11/5/2012 were the longest and heaviest. Total length and total weight covaried (Spearman's correlation coefficient: r=0.735, p<0.001), similarly to *S. abaster* juveniles. The number of dorsal fin rays did not differ between the juveniles of *S. typhle* species (Figure 5.16, Table 5.12).

Within each birth, most offspring were of the same length and weight and had the same number of dorsal fin rays (Figure 5.17), while their coloration varied from a light shade (light green or brown) to a darker (dark green or brown) or even black in a similar pattern as *S. abaster* juveniles. However, data on the exact body color of *S. typhle* juveniles were only recorded for those born in 3/6/2012. Therefore, comparison between the offspring of the three pregnant males was not possible. However, among the juveniles born in 3/6/2012 the dark-colored were longer and heavier (Figure 5.18).

Table 5.12. Total length (TL, mm), total weight (TW, mm) and number of dorsal fin rays (NDFR) among *S. typhle* 1st day juvenile specimens born from three different pregnant males under reared conditions in the present study (*N*, number of individuals; *Mean*, mean value*; Min*, minimum value; *Max*, maximum value*; St.Dev*, standard deviation).
Πίνακας 5.12. Ολικό μήκος (TL, mm), ολικό βάρος (TW, gr) και αριθμός ακτίνων του ραχιαίου πτερυγίου (NDFR) των νεαρών ατόμων (1ης ημέρας) του είδους *S. typhle*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες από τρεις διαφορετικούς αρσενικούς γεννήτορες κατά τη διάρκεια της παρούσας μελέτης (*N*, αριθμός ατόμων; *Mean*, μέση τιμή*; Min*, ελάχιστη τιμή; *Max*, μέγιστη τιμή*; St.Dev*, τυπική απόκλιση).

Date of birth		Mean	Min	Max	St.Dev
6/5/2012 (N=17)	TL	24.3	23.0	28.0	0.11
	TW				
	NDFR				
11/5/2012 (N=33)	TL	27.5	26.0	30.0	0.10
	TW	0.009	0.007	0.010	0.0008
	NDFR	32	32	34	0.57
3/6/2012 (N=78)	TL	26.0	21.0	29.0	0.14
	TW	0.006	0.003	0.009	0.0013
	NDFR	33	30	35	1.22

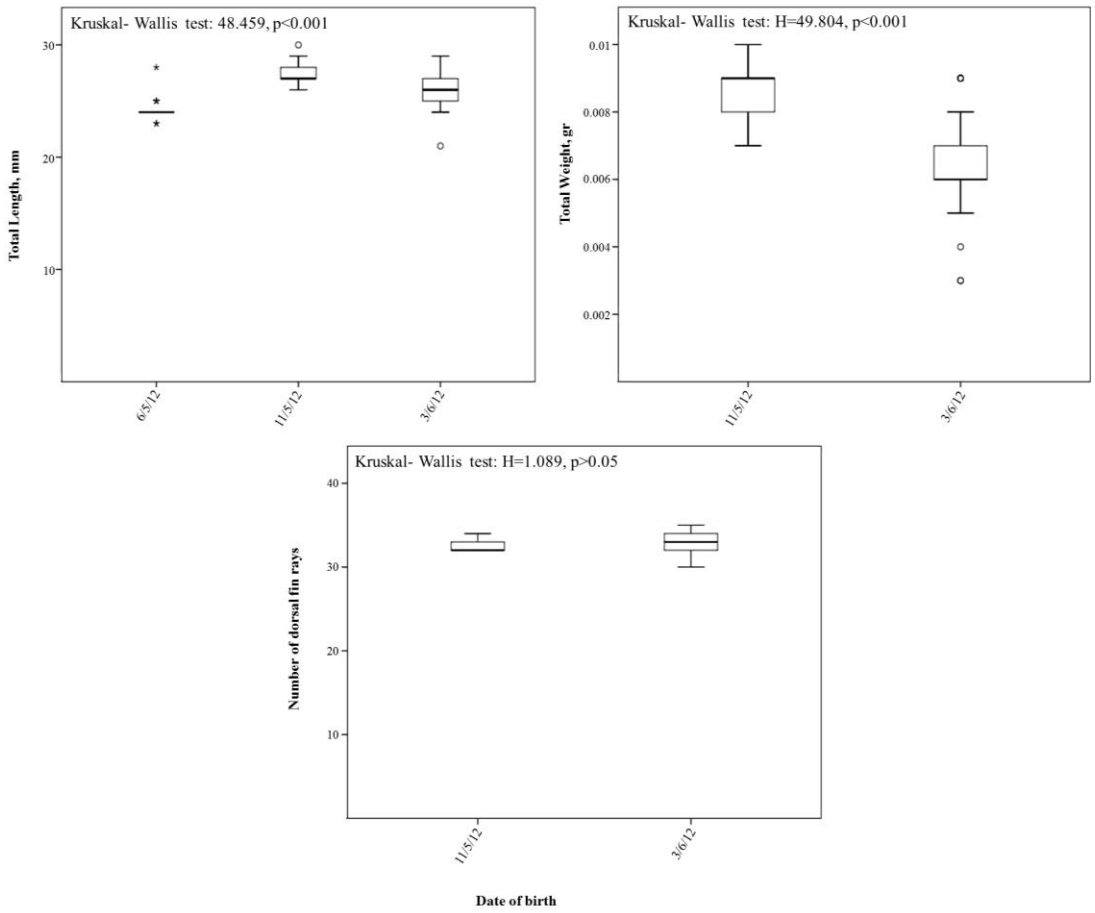


Figure 5.16. Box plots of the total length (TL, mm), total weight (TW, mm) and the number of dorsal fin rays (NDFR) among *S. typhle* 1st day juvenile specimens born from three different pregnant males under reared conditions in the present study (*H,* Value of Kruskal-Wallis non parametric test; *P,* level of significance, the middle bold black line within each box corresponds to the median, the points are outliers, the asterisks are extreme outliers and the T-bars that extend from the boxes correspond to the standard deviation).

Εικόνα 5.16. Θηκογράμματα του ολικού μήκος (TL, mm), ολικού βάρους (TW, gr) και του αριθμού των ακτίνων του ραχιαίου πτερυγίου (NDFR) μεταξύ των νεαρών ατόμων (1ης ημέρας) του είδους *S. typhle*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες από τρείς διαφορετικούς αρσενικούς γεννήτορες κατά τη διάρκεια της παρούσας μελέτης (*H,* η τιμή του μη- παραμετρικού τεστ Kruskal-Wallis; *P,* επίπεδο σημαντικότητας, η μεσαία έντονη γραμμή κάθε θηκογράμματος αντιστοιχεί στο διάμεσο, οι κύκλοι σε ακραίες τιμές, οι αστερίσκοι σε εξαιρετικά ακραίες τιμές και οι γραμμές σχήματος «Τ» στην τυπική απόκλιση του δείγματος).

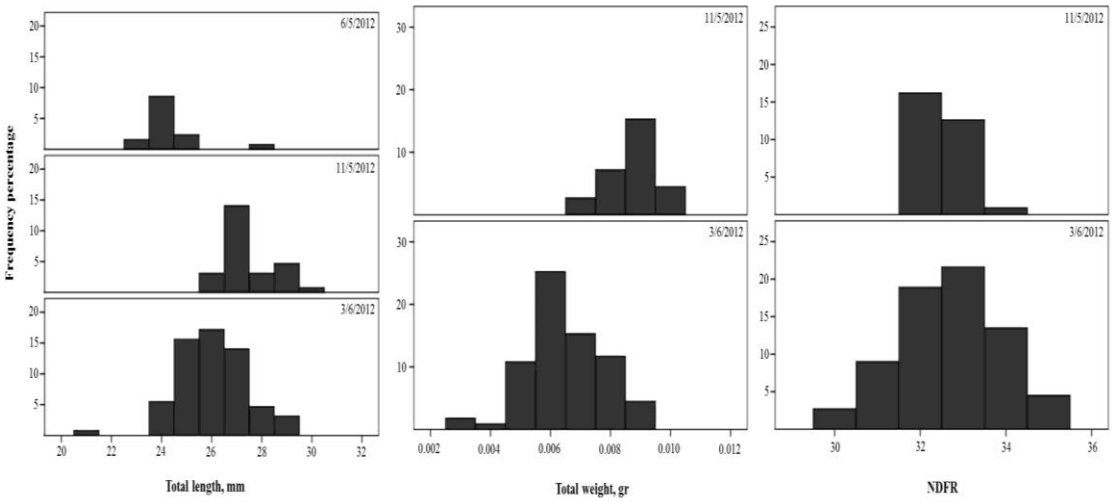


Figure 5.17. Total length, total weight and number of dorsal fin rays (NDFR) distribution of 1st day offspring of *S. typhle* species born under reared conditions in the present study.

Εικόνα 5.17. Κατανομή ολικού μήκους, ολικού βάρους και αριθμού των ακτίνων του ραχιαίου πτερυγίου των νεογέννητων απογόνων (1ης ημέρας) του είδους *S. typhle*, τα οποία γεννήθηκαν υπό εργαστηριακές συνθήκες κατά τη παρούσα μελέτη.

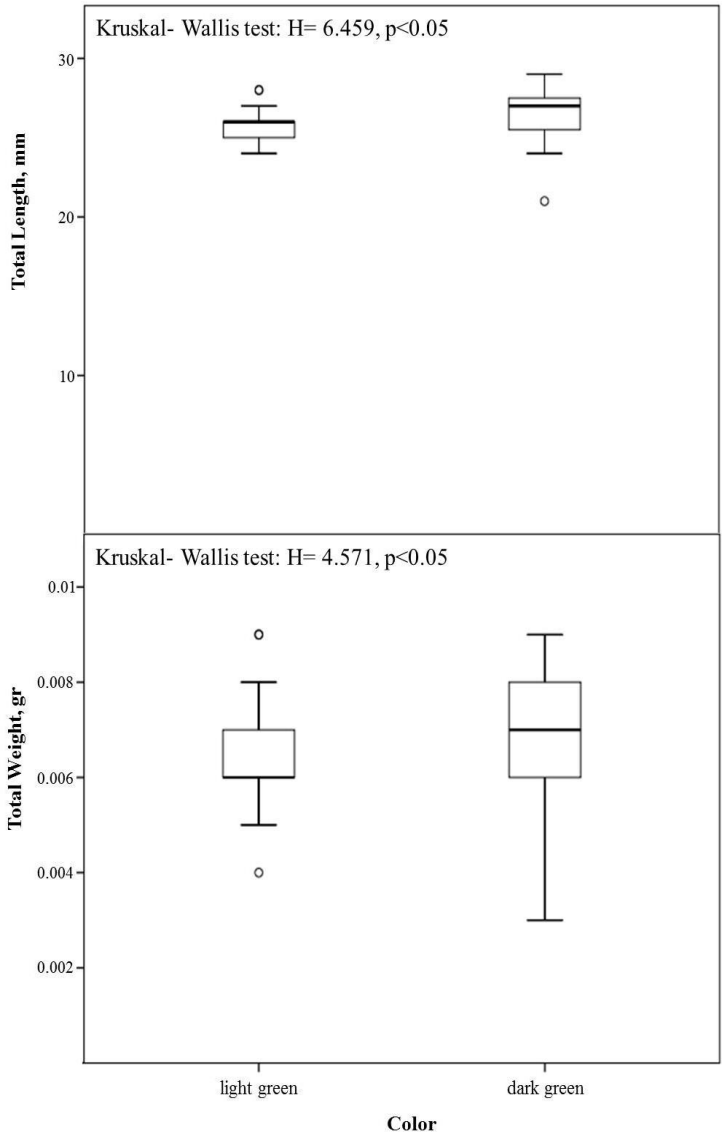


Figure 5.18. Box plots of the statistically important differences in the total length (TL, mm) and number of dorsal fin rays (NDFR) according to the color of the 1st day juveniles of *S. typhle* species, born from three different pregnant males under reared conditions in the present study (*H*, Value of Kruskal-Wallis non parametric test; *P*, level of significance, the middle bold black line within each box corresponds to the median, the points are outliers, the asterisks are extreme outliers and the T-bars that extend from the boxes correspond to the standard deviation).

Εικόνα 5.18. Θηκογράμματα των στατιστικά σημαντικών διαφορών του ολικού μήκος (TL, mm) και του αριθμού των ακτίνων του ραχιαίου πτερυγίου (NDFR) σε σχέση με το χρώμα των νεαρών ατόμων (1ης ημέρας) του είδους *S. typhle*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες από τρεις διαφορετικούς αρσενικούς γεννήτορες κατά τη διάρκεια της παρούσας μελέτης (H, η τιμή του μη- παραμετρικού τεστ Kruskal-Wallis; P, επίπεδο σημαντικότητας, η μεσαία έντονη γραμμή κάθε θηκογράμματος αντιστοιχεί στο διάμεσο, οι κύκλοι σε ακραίες τιμές, οι αστερίσκοι σε εξαιρετικά ακραίες τιμές και οι γραμμές σχήματος «Τ» στην τυπική απόκλιση του δείγματος).

5.3.2.3. Parentage analysis and genetic mating system of *Syngnathus typhle* species

The three pregnant males, a subset of their offspring (N=78) and eighteen individuals of the wild population (WP) were successfully genotyped at each of the four loci (Table 5.3 in Appendix). All loci were variable, as the number of alleles per locus varied from eighteen at S.abas9 (14 at WP) to twenty one at S.abas4 and 7 (16 and 20 at WP respectively) (Table 5.13). The average expected heterozygosity over loci was 0.95 (ranging from 0.93 at S.abas4 to 0.95 at S.abas3, 7 and 9) and the mean polymorphic information content (PIC) value was 0.91 (ranging from 0.90 at S.abas4 to 0.92 at S.abas4). The high values of both indices revealed that these markers are highly informative for paternity estimation (Botstein et al. 1980). Significant deviation from HWE at a 0.05 α-level was not detected in any loci (Table 5.4). The presence of null alleles was detected at S.abas3, but they were rare at the other three loci. Their occasional presence would not change the results because most individuals were heterozygous for the genotyped alleles as revealed by PIC and expected heterozygosity values.

Table 5.13 Genetic variability of *S. typhle* Drepano population at 4 microsatellite loci used for parentage analysis as revealed by the allele frequency, heterozygosity values, Hardy-Weinberg test and polymorphic information content for each locus of the wild population genotyped in the present study (*NA*, number of alleles found in offspring and wild population; *, alleles found in offspring; F_A, allele frequency in the wild population; *k* number of alleles found in the wild population; *N*, number of individuals genotyped per locus; H_o, observed heterozygosity; H_e, expected heterozygosity; *PIC*, polymorphic information content; *HW*, values of Hardy-Weinberg test; F_N, null allele frequency; *NS*, non-significant).

Πίνακας 5.13. Γενετική ποικιλομορφία του είδους *S. typhle* στον πληθυσμό του Δρεπάνου στους 4 μικροδορυφορικούς τόπους που χρησιμοποιήθηκαν στον έλεγχο της πατρότητας. Το επίπεδο της ποικιλομορφίας προκύπτει από την συχνότητα των αλληλομόρφων, τον έλεγχο της ισορροπίας Hardy-Weinberg, τον δείκτη πολυμορφισμού των μικροδορυφορικών τόπων και ετεροζυγωτίας σε κάθε τόπο του άγριου πληθυσμού του είδους στη παρούσα μελέτη (*NA*, ο αριθμός των αλληλομόρφων του άγριου πληθυσμού και των απογόνων, *, αλληλόμορφα απογόνων; F_A, συχνότητα των αλληλομόρφων του άγριου πληθυσμού; *k*, ο αριθμός των αλληλομόρφων του άγριου πληθυσμού; *N*, αριθμός ατόμων που γενοτυπήθηκε ανά τόπο; H_o, παρατηρούμενη ετεροζυγωτία; H_e, αναμενόμενη ετεροζυγωτία; *PIC*, δείκτη πολυμορφισμού των μικροδορυφορικών τόπων; *HW*, τιμές του ελέγχου ισορροπίας Hardy-Weinberg; F_N, συχνότητα μηδενικών αλληλομόρφων; *NS*, μη στατιστικά σημαντικό).

				Wild population						
Locus	**NA**	**Allele**	**F_A**	**k**	**N**	**H_o**	**H_e**	**PIC**	**HW**	**F_N**
S.abas3	19	178*	0.03	16	15	0.67	0.95	0.92	NS	0.16
		186*	0.03							
		198*	0.07							
		202*								
		206*	0.03							
		210*								
		214*	0.07							
		218*	0.07							
		222*	0.13							
		226*	0.03							
		230*								
		234*	0.10							
		238*	0.07							
		242*	0.07							
		246*	0.03							
		254*	0.03							

		258*	0.10							
		262*	0.10							
		294*	0.03							
		193*								
		197*	0.03							
		205*	0.03							
		209*								
		213*	0.06							
		217*	0.06							
		221*	0.03							
		225*	0.08							
		229*	0.19							
		233*	0.11							
S.abas4	21	237*	0.11	16	18	0.94	0.93	0.90	NS	-0.02
		241*								
		245*								
		249*	0.08							
		253*	0.08							
		257*	0.03							
		273*								
		279*	0.03							
		345*	0.03							
		437*	0.03							
		497*	0.03							
		198*	0.03							
		210*	0.03							
		218*	0.03							
		222*	0.06							
		226*	0.03							
		230*	0.03							
		234*	0.11							
		238*	0.03							
		242*	0.03							
		246*	0.08							
S.abas7	21	250*	0.08	20	18	1000.00	0.95	0.92	NS	-0.04
		254*	0.08							
		258*	0.14							
		262*	0.08							
		266*								
		270*	0.03							
		278*	0.03							
		286*	0.03							
		302*	0.03							
		454*	0.03							
		498*	0.03							
		192*								
		196*	0.08							
		200*	0.12							
		204*	0.04							
		208*								
S.abas9	18	212*	0.04	14	13	0.92	0.95	0.91	NS	0.00
		216*	0.08							
		220*								
		224*	0.12							
		228*	0.08							
		232*	0.08							

	236*	0.08	
	240*	0.04	
	244*	0.04	
	248*	0.08	
	256*	0.12	
	268*	0.04	
	272*		

Colony software assigned all embryos to their known father without any mismatches. Thus, there is complete confidence of paternity of the broods in each *S. typhle* male, similarly to *S. abaster*. In total, at least 7 females contributed to the broods of pregnant males (Table 5.4 in Appendix). Similarly to *S. abaster*, this is the minimum number of expected female donors. Two of the three males received eggs from multiple females while one male received eggs only from one. The mean number of successful mates per male was 2.33 (St.Dev=1.15) ranging from one to three mates (Table 5.14). Within each birth, the number of offspring sired by mothers varied from one (mothers ID: #5) to twenty nine (mother ID: #7) (Table 5.14). Contrary to *S. abaster* species, no sign of females multiple mating was noted since no offspring of different males with identical mother genotypes were found (Table 5.14).

Table 5.14. Description of the mating system of the three pregnant males of *S. typhle* species that gave birth under laboratory conditions and were used for parentage analysis in the present study. The number of possible female donors and the number of their sired juveniles was estimated by the Colony software.
Πίνακας 5.14. Περιγραφή του αναπαραγωγικού συστήματος των τριών κυοφορούντων αρσενικών ατόμων του είδους *S. typhle*, τα οποία γέννησαν σε εργαστηριακές συνθήκες και χρησιμοποιήθηκαν στην ανάλυση μητρότητας. Ο αριθμός των πιθανών θηλυκών γεννητόρων και των απογόνων τους υπολογίστηκε από το πρόγραμμα Colony.

Date of birth	Father ID	Number of juvenile	Genotyped juveniles	Possible mothers	Mother ID	Number of sired juveniles	Shared mothers
6/5/2012	G13	19	19	3	#4 #5 #6	6 3 10	0
11/5/2012	G6	39	29	1	#7	29	0
3/6/2012	G5	78	29	3	#1 #2 #3	10 13 6	0

The effect of the different mothers on the total length and number of dorsal fin rays of their offspring was not estimated as the pairing of the genotyped offspring and the original data set was not possible.

5.3.3. Morphometric and meristic analysis comparison between 1st day juveniles of *Syngnathus typhle* and *Syngnathus abaster* species

In overall, *S. typhle* juveniles were statistically longer, heavier and had more dorsal fin rays than *S. abaster* (Figure 5.20).

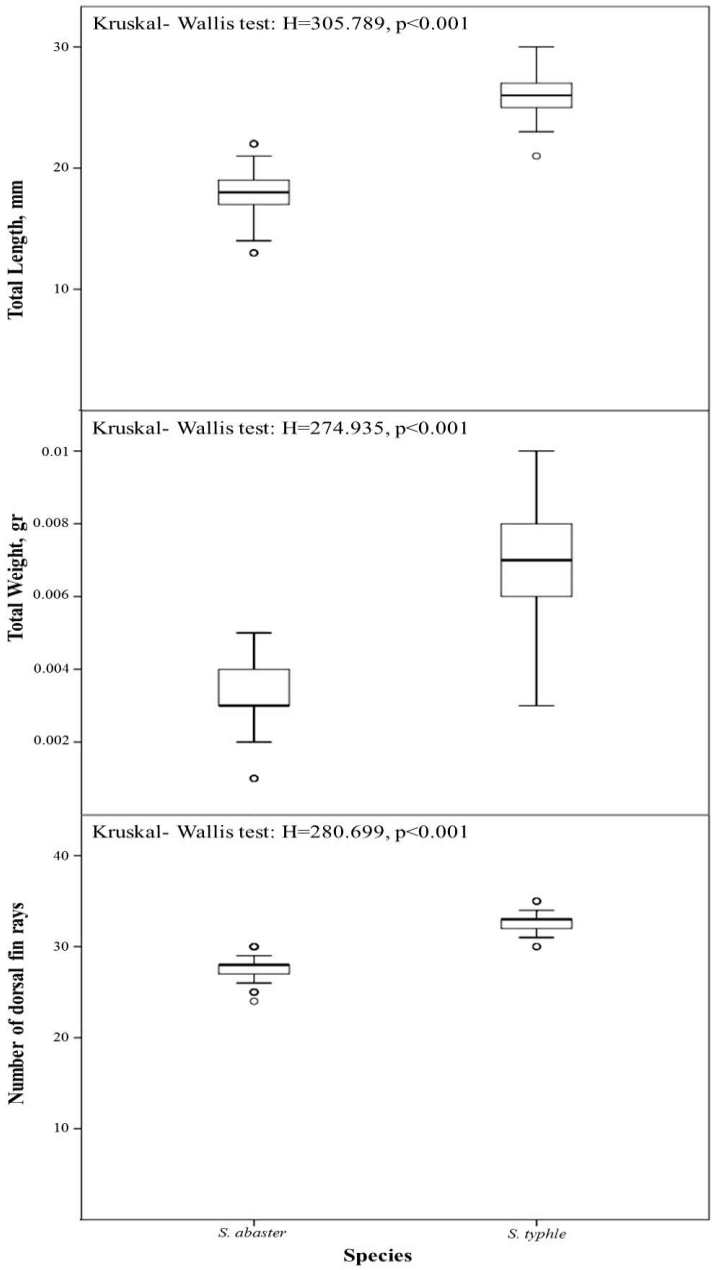


Figure 5.19. Box plot of the total length (TL, mm), total weight (TW, gr) and number of dorsal fin rays of *S. abaster* and *S. typhle* 1st day juvenile specimens born under reared conditions in the present study (*H*, Value of Kruskal-Wallis non parametric test; *P*, level of significance, the middle bold black line within each box corresponds to the median, the points are outliers and the T-bars that extend from the boxes correspond to the standard deviation).

Εικόνα 5.19. Θηκόγραμμα του ολικού μήκος (TL, mm), ολικού βάρους (TW, gr) και του αριθμού των ακτίνων του ραχιαίου πτερυγίου των νεαρών ατόμων (1ης ημέρας) των ειδών *S. abaster* και *S. typhle*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες κατά τη διάρκεια της παρούσας μελέτης (*H*, η τιμή του μη- παραμετρικού τεστ Kruskal-Wallis; *P*, επίπεδο σημαντικότητας, η μεσαία έντονη γραμμή κάθε θηκογράμματος αντιστοιχεί στο διάμεσο, οι κύκλοι σε ακραίες τιμές και οι γραμμές σχήματος «Τ» στην τυπική απόκλιση του δείγματος).

Table 5.15. Descriptive information on the individuals that were used in the morphometric comparison between 1st day juveniles of *S. abaster* and *S. typhle* species born under reared conditions from different males during the present study *(N,* number of individuals).
Πίνακας 5.15. Περιγραφικά στοιχεία των ατόμων που χρησιμοποιήθηκαν στη μορφομετρική σύγκριση των νεαρών ατόμων (1ης ημέρας) των ειδών *S. abaster* και *S. typhle*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες κατά τη διάρκεια της παρούσας μελέτης *(N,* αριθμός ατόμων).

Species	Date of birth	N
S. abaster (N=278)	14/5/2012	39
	29/5/2012	56
	2/6/2012	55
	7/6/2012	39
	3/7/2012	32
	9/7/2012	34
	13/7/2012	23
S. typhle (N=92)	11/5/2012	32
	3/6/2012	60

A subset of 1st day juveniles of *S. abaster* (n=278) and *S. typhle* (n=92) species were examined morphometricaly (Table 5.15).

The PCA extracted 26 components (Table 5.7 *in* Appendix). The first PC (PC I) accounts for 64.1% of total variance and is characterized by shape changes of snout, main body and caudal fin (elongation) as well as trunk (shortening) along the x- axis. The second PC (PC II) accounts for 16.4 % of total variance and depicts the corresponding body changes along the y-axis. The rest of the PCs explain 19.5% of the total variance and describe aspects of intraspecific shape variation (Table 4.5 *in* Appendix). The PC scores were used as variables in the Linear Discriminant Analysis (LDA) to assess interspecies differences among newborn individuals of *S. abaster* and *S. typhle* species.

The structure of between-species shape variation was assessed by Linear Discriminant Analysis (LDA) of partial warps and uniform components. One Linear Function (LF 1) was extracted (Mahalanobis distance= 14.99, $p<0.001$). Species pair difference (in both Procrustes and Mahalanobis scale) was significant under permutation tests ($p < 0.0001$). The cross-validation test (leave-one-out method) from LDA correctly classified all cases (100%) proving that the two species were completely separated (Table 5.16).

Therefore, LDA showed a considerable difference among the 1st day juveniles of the two species, defining two non-overlapping groups: i) *S. typhle* species and ii) *S. abaster* species. The shape changes associated with the LF axis showed that *S. typhle* specimens had more elongated snout, elongated and thinner main body, whereas their trunk was shorter compared to *S. abaster* (Figure 5.20).

Table 5.16. Classification results for the cross-validation procedure of *S. abaster* and *S. typhle* 1st day juvenile specimens born under reared conditions in the present study, using principal components of shape scores as variables in a linear discriminant analysis.
Πίνακας 5.16. Επανατοποθέτηση των νεαρών ατόμων (1ης ημέρας) των ειδών *S. abaster* και *S. typhle*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες κατά τη διάρκεια της παρούσας μελέτης, βάσει της διαχωριστικής ανάλυσης.

Species	**Predicted group membership**		
	S. abaster	*S. typhle*	*Total*
Count			
S. abaster	92	0	92
S. typhle	0	278	278
Percent %			
S. abaster	100.0	0.0	
S. tyhle	0.0	100.0	

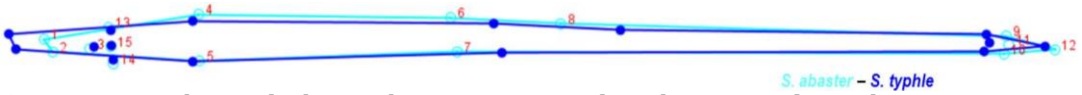


Figure 5.20. Observed shape changes associated with scores along the Linear Discriminant Analysis axis of *S. abaster* and *S. typhle* 1st day juvenile specimens born under reared conditions in the present study The wireframes depict icons for deviations (dark blue line) from the corresponding mean shape for juvenile individuals of *Syngnathus* species (light blue line).
Εικόνα 5.20. Παρατηρούμενη αλλαγή του σχήματος του σώματος με βάση τις τιμές του άξονα της Γραμμικής Διαχωριστικής Ανάλυσης των νεαρών ατόμων (1ης ημέρας) των ειδών *S. abaster* και *S. typhle*, τα οποία γεννήθηκαν σε εργαστηριακές συνθήκες κατά τη διάρκεια της παρούσας μελέτης. Το πλέγμα απεικονίζει την απόκλιση (σκούρα μπλε γραμμή) από το σχήμα αναφοράς των νεαρών ατόμων των δύο ειδών του γένους *Syngnathus* (ανοιχτή μπλε γραμμή).

5.4. DISCUSSION

5.4.1. Male pregnancy and parturition

The present chapter described for the first time the male pregnancy and parturition process of *S. abaster* and *S. typhle* species. Even though both species are characterized by an "inverted" brood pouch, differences and similarities in the procedures were found.

During pregnancy the brood pouch of the two species changes both in color and texture. From a stiff structure with similar color to the rest of the body in the beginning of the pregnancy, it became dark colored and soft before parturition. These differences in the external morphology of the pouch are attributed to the occurring embryonic and larval developmental stages. The pouch of the two species is semi-transparent; more transparent in *S. abaster* and less in *S. typhle*. In the beginning of the pregnancy the transparent or light-yellow inseminated eggs of both species (Kornienko 2001, Silva et al. 2006a) have no impact on the color of the pouch. In *S. abaster* embryos the first pigmented cells appear in the head region within the first week of the development and afterwards are found in the antero-posterior embryonic axis (Silva et al. 2006a). As the pigmentation of the embryos evolves, it has a visible effect on the color of the pouch. Towards the end of the gestation the larvae are almost fully formed and ready to be released. This means that their body pigmentation is complete too, causing the dark color of the pouch.

Besides the color, the texture and the size of the pouch also changes along the pregnancy. As already mentioned the pouch of pipefish composes of fleshy bilateral folds that meet on the ventral midline of the pouch and enclose the eggs (Herald 1959; Wilson et al. 2001; Silva et al. 2006). After transfer, eggs are surrounded by an epithelium rich in blood vessels, which provides nutrition by osmosis until the yolk sack of the larvae is absorbed; pseudo-placenta (Carcupino et al. 1997; 2002). Apart from the epithelium, the connective tissue layer of the pouch that supports the pseudo-placenta expands and becomes porous (Theverin 1936 according to Kornienko 2001). In the late stages of larva development (when the larva develop to juveniles ready to be released), this structure gradually shrinks and the folds of the marsupium must unravel to release the offspring.

In the beginning of the pregnancy, the eggs and the pseudo-placenta thicken the marsupium. As pregnancy proceeds, the embryos hatch and the thick layers of the eggs are dismissed. The development of fully formed embryos and the shrinking of the placenta make the folds of the pouch softer, enabling the formation of a fissure from which the embryos will be released. The change in size of the pouch is due to the expansion of the assortative connective tissue. Observations on seahorses showed a similar pattern (Lockwood 1867, according to Kornienko 2001): "*After spawning the pouch walls thicken and turn porous, whereas after the fries are "born" the pouch is flabby and hangs like a thin membrane*". Therefore, developmental stages and structural changes occurring into the pouch have direct impact on its external morphology, visible even in the semi-transparent marsupium of *S. abaster* and *S. typhle*.

The pouch of the species of the genus *Syngnathus* varies from transparent (Paczolt and Jones 2010) to semi-transparent (Jones et al. 2010). In species with transparent marsupium such as the gulf pipefish developmental stages are easily distinguished with observation of the pouch i.e. *Syngnathus scoveli* species. In *S. abaster* and *S. typhle* direct observation of the stages through the pouch is not so easy (especially in *S. typhle*). However, the texture, the size and the color of the marsupium could be a good indicator. A stiff and similar to the body colored pouch is an indication of early developmental stages, most possible within the first two weeks. A moderately stiff pouch with a small deviation from body color is a sign of mid-pregnancy stage. The more expanded, black colored and soft the pouch gets, the more far along the pregnancy is and the time of parturition gets closer. Such an empirical estimation of the embryonic and development stages could be helpful in reproductive biology field studies. By observation and without sacrificing the fish, the researcher acquires on the spot an overview of an individual's –and even a whole population's- reproductive period timeframe.

The process of parturition and especially the contractions must be very stressful and painful for both species as indicated by the bending and twisting moves of the body. During the contractions, *S. typhle* males retained their pelagic position. In the same period- contrary to our expectations- all males of *S. abaster* species exhibited a temporal shift from the normal bottom dwelling position to a more upright. Therefore, during the actual process of parturition the difference in their vertical position was not retained, indicating a difference in the species behavior. Sharp movements of the body during birth have been also recorded in the seaweed pipefish, *S. schlegeli* and in the opossum pipefish *M. brachyurus* (Watanabe and Watanabe 2002; Frias-Torres 2004). Both of these species are benthic, similar to *S. abaster*. However, changes in their vertical position were not recorded as males remained close to the bottom, in a diagonal head-up orientation during the whole process (Watanabe and Watanabe 2002; Frias-Torres 2004). Therefore, this temporal upright vertical distribution of *S. abaster* seems not to be a common behavior among benthic syngnathids. Intense contractions could be a possible cause for the observed swift. However, additional data are required before final conclusions are made.

Most of the males of both species released their embryos in the night or early in the morning in consecutive sporadic batches. The first and the last batches composed of a few individuals. The majority of the offspring were born somewhere in the middle of the procedure in one large or two smaller batches. The intermittent contraction ensured that the pseudo-placenta will be properly detached from inner walls of the pouch and free the offspring. Silva et al. (2006a) observed that *S. abaster* gave birth, during a 2–3 day period. However, in the present study brood release never lasted more than 24 hours.

The dusk or nocturnal preference for offspring release must be a strategy to maximize offspring survival. More particularly, in nature, night spawning might be preferred as it helps avoiding predators for both newborns and parents- most such predators are inactive during that period (Helfman 1993, Fujita and Kohda 1998). At dusk, the risk of predation is lower for newborns but higher for adults. In such case, parents'

survival is jeopardized in favor of maximizing survival of the offspring (Helfman 1993). For the same reasons, nocturnal preference was also reported in the seaweed and opossum pipefishes (Watanabe and Watanabe 2002; Frias-Torres 2004), in the scorpionfishes, *Sebasticus marmoratu* and *Sebastes flavidus* (Eldreidge et al. 1991, Fujita and Kohda 1998) and in many oviparous pelagic fish species (e.g. Johannes 1978).

The sporadic release of the embryos and prolonged total duration of the parturition could also be a strategy to maximize offspring survival. As juveniles exit the pouch in batches, not all of them risk being "lost" in case of predation. However, the prolonged parturition exposes males to predation, as due to the contractions they cannot stay hidden in the vegetation. This is probably the reason why in the seaweed and opossum pipefishes brood release event lasted a few hours (Watanabe and Watanabe 2002, Frias-Torres 2004). Such a long period of contractions (up to 12 h) was only recorded in the seahorse brood (*Hippocampus sp.*), with the actual release lasting just a few seconds (Garrick-Maidment 1997, according to Frias-Torres 2004). Nocturnal or duck preference and sporadic release probably compensate for the prolonged parturition period of *S. abaster* and *S. typhle*.

S. abaster and *S. typhle* juveniles were released from the marsupium fully developed, with absorbed yolk sack and- as expected- immediately adopted benthic and pelagic behavior, respectively (Vincent 1992; Ahnesjö 1992; Silva et al. 2006a). In rare cases when the male gave birth under stressed conditions, premature embryos were born (the yolk sack was obvious) but they were not viable. Therefore, under normal conditions newborns of both species do not undergo a larval stage. This is due to the fact that, the interaction between the male brood structure and the developing embryos is inversely proportional to the degree of egg exposure to the external environment (Carcupino et al. 2002). Most species of the genus *Syngnathus* and all seahorses have elaborate brood pouch (inverted and fully closed type) thus releasing fully formed embryos.

5.4.2. Genetic mating system

5.4.2.1. *Syngnathus abaster* species

Pregnant males of *S. abaster* gave birth to an average number of 43 eggs. The number of offspring is within the range of those reported by Silva et al (2006a) (37 ± 11 eggs) but larger than those reported by Hubner et al. (2013) (24 eggs) and Cunha (2012) (27.58 eggs). This difference could not be attributed to males' size as; i) it did not differ between the males and ii) the number of offspring was not correlated to size of males in the Drepano population.

The mean number of successful mates per male was 4.25 ranging from three to six. This is a substantial difference form the values of 2.8 (ranging from two to four female mates) and 2.82 (ranging from two to three) reported in the studies of Hubner et al. (2013) and Cunha (2012), respectively. This difference could be even bigger, as the estimated 28 mothers in the present study is the minimum number of the female donors. The high number of successful mates per male recorded in the present study could have possibly caused the difference of the clutch size mentioned above, but a larger data set is needed in order to test this hypothesis.

Besides multiple mating, the occurrence of different males' offspring with identical mothers (mother id #3, #13, #16, #17, #21) was noted. As the genotypes of these females were reconstructed and not subtracted from the wild population pool, each genotype could either belong to one female mating multiple or to different females with identical genotype, mating with only one male each time. Mothers with ID, #16, #17 and #21 sired juveniles in two broods that were born in close dates and thus were inseminated in the same period, more or less. On the contrary, mothers #3 and #13 sired embryos in two and three broods respectively, which were born almost two months apart and thus were inseminated in different periods. Given that the same female in Ria de Aveiro (Portugal) population was found to lay eggs on different males within a ten day period (Silva et al. 2009), we believe that mothers #16, #17 and #21 correspond to one individual each time mating multiple with different males.

However, things were more complicated for mothers #3 and #13, as they could either be one female mating in two different periods or different females with identical genotype. *S. abaster* females are batch spawners, whose eggs mature continuously, and as long as they have ripe ovaries, they can mate at any given time during the reproductive period (Silva et al. 2009). Thus, if these females were not able to mate within a short period, they would keep searching for males without having a strict time frame in which they must mate. In such a case, the above mentioned mothers mated multiple times with different males within two months. On the other hand, the alleles of these two mothers' genotypes were present in the wild population (allele frequencies ranging from 0.06-0.012 at the four loci). Due to the Mendelian way of microsatellite inheritance (Turnpenny and Ellard 2005), there is a possibility- even though a small one- for two individuals to share

the exact same alleles. Therefore, the possibility of different females with the same genotype cannot be excluded for these potential mothers.

Even though it cannot be absolutely sure whether the mothers in question were the same individual –each time- or different with the same genotype, the result of the present study indicated that males mated with more than one female and females possibly mated with more than one male. Therefore, the genetic mating system of Drepano population is most likely characterized as polygynandrous. This result is in accordance with the social and genetic mating system observed in Portuguese populations (Silva et al. 2006b; Cunha 2012; Hubner et al. 2013).

However, as already mentioned the degree of male multiple mating was higher in the population of Drepano than the value estimated in the studies of Cunha (2012) and Hubner et al. (2013). This outcome did not come as a surprise, as males multiple mating was found to differ among isolated populations of syngnathids (Rispoli and Wilson 2008; Mobley and Jones 2007; 2009) or other taxa (Weatherhead and Boag 1997; Griffith *et al*. 1999; Soucy and Travis 2003; Durrant and Hughes 2005). Even though the genetic distance between the populations from N. Ionian Sea and Portuguese lagoons was not directly estimated, the species isolation by distance isolation pattern at a regional and Mediterranean scale (Chapter 2, Alaya et al. 2011; Sanna et al. 2013a) is a strong indicator for the separation of the two populations. Thus, genetic isolation most likely affects male multiple mating in *S. abaster* species.

Rispoli and Wilson (2008) and Mobley and Jones (2009) indicated a negative correlation between male mating intensity and sexual size dimorphism across a wide range of *S. typhle* and *S. floridae* species distribution, respectively. The higher number of possible mates for males in Drepano station (absence of sexual size dimorphism in the population) compared to the studies of Cunha (2012) and Hubner (2013) (moderate levels of sexual size dimorphism) indicates the impact of sexual selection on the degree of males multiple mating. This difference could be also contributed to environmental factors (such as temperature, population density, predation etc.), too. However, more data are required by reaching any final conclusion.

Apart from the impact of sexual selection and geographical isolation on the levels of male polygamy, two more aspects need to be discussed regarding the *S. abaster* mating system. First of all the total length, weight and number of dorsal fin rays of offspring varied between and within the broods. However, given that the only available data on the possible mothers is their genotype, it cannot be decided whether it is the father, the mother or a combination of the two of them that mostly affected these differences.

Furthermore, surprisingly, in almost all males, at least one female sired only one or two of the genotyped offspring. This is the first time such a pattern is found in *S. abaster* species, while it has been reported before in *S. typhle* (Jones et al. 1999). Reasons for this outcome remain uncertain. Given that the process of mating is time and energy consuming

it is unlikely that both sexes would be involved in courtship just to allocate or accept such small number of eggs. However, in the wild it has been observed that mating events are often interrupted (Silva et al 2009) by i) the presence of a second "jealous/intruding" female- and more rarely male- or ii) the re-evaluation of mates in the begging of the reproductive event (Vincent et al. 1995; Silva et al. 2009). A second possibility is that the small numbers of eggs sired to few females could be the result of brood reduction. During male pregnancy the development of some eggs is terminated. (Jones et al. 1999). In particular, it is recorded that the number of released offspring is lower than the number of received eggs (Ahnesjo 1992). Even though undeveloped eggs are dispersed in the pouch, there is a possibility that they are connected to the quality of female donors (Ahnesjo 1995) and this could lead to reduced sires from some mothers.

The fact that this small number of sired offspring by some females has not been reported so far in *S. abaster*, implies a methological artifact of previous studies. As females deposit unfertilized eggs into a brood pouch, eggs from the same mother are packed together (Silva et al 2006b). Therefore, in previous studies the pouch was divided into "n" equal zones (according to the number of embryos within the pouch) and then a representative sample from each zone was genotyped. With this subtractive method at least one embryo from each female was expected to be genotyped, minimizing though the actual number of the analyzed embryos compared to the total number (e.g. Mobley and Jones 2007; 2009; Hubner et al. 2013). However, if there are indeed females contributing with one or two eggs in the brood they can easily be lost with this subtractive method. In the present study the genotyped embryos varied from almost 50% to 93% of the released offspring, raising thus the possibility to detect mothers with such small number of sired offspring. This could possibly be one more reason for the higher degree of males multiple mating found in the present study.

5.4.2.2. *Syngnathus typhle* species

Pregnant males of *S. abaster* gave birth to an average number of 43 eggs. This number is lower than most of the examined populations (except from the Portuguese) in Rispoli and Wilson (2008) study, while it is in the same levels with the Swedish population in Jones et al. (1999). Similarly to *S. abaster* species, this difference could not be attributed to males' size as; i) it did not differ between the populations and ii) the number of offspring was not correlated to males size in Drepano population. The low number of released offspring must be associated to the fact that the studied males had mated in the beginning of the reproductive period. It has been shown that the number of embryos a male can brood peaks in the middle of the reproductive season and decreases in the last months (Berglund et al. 1986). Therefore, if a more representative sample of the population was included, the results may have been different.

The mean number of successful mates per male was 2.33 ranging from one to three females. This is a substantial difference from the value of 3.7 and 1.3 mates per male in the Nord Sea and Venice lagoons population, but only a slight one when comparing Drepano population to the ones from the Ria Fermossa lagoon (Portugal) and Biscay Gulf (2.9 and 2.4 females respectively) (Rispoli and Wilson 2008).

Female multiple mating was not recorded in the present study. As already mentioned the studied males had mated in the beginning of the reproductive period and corresponded to a minor portion of the breeding population. Therefore, if more broods were studied, the results of males multiple mating may have been different. Similarly, it seems that females may have mated with additional males which were not collected. Thus, the results probably severely underestimate the true rate of the species actual levels of multiple mating. Before deducing any final conclusions on the species mating system in Drepano population more brooding males need to be examined. Similarly, the impact of sexual size dimorphism and geographic isolation cannot be confidently estimated.

A difference in the total length and weight, but not in the number of dorsal fin rays was recorded between and within the offspring of the three different broods. Once again, the absence of data on the size of the possible mothers does not allow the deduction of safe conclusions. Contrary to *S. abaster* species, no mother with less than three sired offspring was detected. Even in the brood that only three offspring were sired to one mother, all the released juveniles of that brood were genotyped in order to rule out the possibility of undetected sired newborns.

5.4.3. Comparison of *Syngnathus abaster* and *Syngnathus typhle* juveniles

Analysis of size (total length and total weight), shape (morphometric variables), anatomical traits (number of dorsal fin rays), as well as observations on the behavior (vertical position), showed that *S. abaster* and *S. typhle* newborns were distinct. First of all, as expected, *S. typhle* juveniles were longer and had more dorsal fin rays than *S. abaster*. Also, morphometric analyses showed they had a more elongated snout and main body, but a shorter trunk compared to *S. abaster*. The juveniles of the two species were also spatially segregated with *S. abaster* newborns acquiring a benthic position from the moment they were born while *S. typhle* swam in the upper part of the aquarium.

However, these differences are also, distinct traits between adult specimens of the two species (Dawson 1986, www.fishbase.org). The fact that major interspecies differences -following the adult specimens pattern- were detectable from the first day juveniles indicated that *S. abaster* and *S. typhle* newborns are fully formed individuals, closely resembling adult fish. Silva et al. (2006a) and Sommer et al. (2012) also support that *S. abaster* brooding males give birth to fully formed individuals. However, their findings were based on behavioral (distribution in the water column), qualitative observations (coloration) and meristic characters (dorsal fin rays, body rings). Therefore, this is the first study to statistically prove that size, shape anatomical and behavioral differences between the *S. abaster* and *S. typhle* species are visible from the day they are born.

A final trait of the two species that proves their resemblance to adult specimens was a similarity rather than a difference. The color pattern of both newborns escalated from light green/brown to dark green/ brown or even black. Syngnathids coloration depends on the environment that they live and more particularly on the type and density of vegetation they mimic (Dawson 1986). As the ecosystem of Drepano is a complex one with patchy seagrass and bare sand, adult specimens showed a variety of colors similar to the ones recorded in the juveniles. Thus the pigmentation of juveniles is probably completed before they leave the pouch. An interesting fact is that Silva et al (2006a) recorded all of *S. abaster* offspring having the same dark brown color. Since there are no data on the color of the adult specimens we cannot be sure whether this is a random effect or an actual difference in the juveniles of the two localities.

Post-released morphological data that support the strong resemblance of adult and new born specimens – although rare- were recorded in other syngnathid species too. Three seahorse species, *Hippocampus kuda*, *H. abdominalis* and *H. ingens* and a pipehorse, *Syngnathoides biaculeatus*, were proven to give birth to fully formed individuals (Watson and Sandknop 1996; Gomon and Neira 1998; Mi et al 1998; Dhanya et al. 2005; Choo and Liewe 2006). On the contrary, *Nerophis lumbriciformis*, *N. ophidion* and *Entelurus aequoreus* released smaller and less developed larvae, with primordial fin, present yolk sack and transparent coloration (Russell 1976; Monteiro et al. 2003).

The main factor that determines the developmental stages of released individuals is the type of the brood pouch (Herald et al. 1959). Embryonic development in the syngnathids' inverted or fully closed brood pouch occurs under relatively stable and protected conditions, unaffected of the external environment. Along with the function of mechanical protection, the pseudo-placenta in the marsupium supplies developing embryos with a certain amount of nutritive substances (Mi et al 1998; Ripley et al. 2006; 2009; Kvarnemo et al. 2011). This enables the embryo to efficiently use the yolk throughout the embryonic development and absorb it just before the release of the fully formed fry (Mi et al. 1998; Silva et al. 2006a; Sommer et al. 2012). On the other hand, marsupium-lacking species, such as *N. ophidion* and *N. lumbriciform*is cannot nourish and protect their embryos so effectively. This leads to the release of small planktonic larvae, with little resemblance to adult individuals (Monteiro et al. 2003).

Chapter 6. Conclusions

The aim of the present study was to examine the biology, the genetic and phenotypic structure and the mating system of the sympatrically occurring syngnathids *Syngnathus abaster* and *Syngnathus typhle* along the Greek coastline. The outcomes of the present thesis showed some remarkable similarities and striking differences between the two species.

In regard to the biology of *S. abaster* and *S. typhle* (Chapter 2), both species seem to have established breeding non-competing populations in Drepano and Neochori ecosystems. The lack of difference in the population structure, growth pattern and reproduction period of both species in the examined stations indicated absence of local adaptations. Therefore either the two ecosystems are not as distinct as they were supposed of being or local adaptations are obvious in a broader geographical range than the studied ones. Therefore, a future perspective would be to examine the biology of the two species at an Ionian- Aegean scale. The different abiotic and biotic factors prevailing in the two Seas (Coll et al. 2010, Bianchi et al. 2012) could potentially be strong drives for differentiation. Also local adaptations could also be present between individuals inhabiting different types of ecosystems i.e. open sea and lagoon. In particular Baker and Foster (2002) revealed a salinity dependent fluctuation in the life-history traits of the threespine stickleback, *Gasterosteus aculeatus* L (Gasterosteiformes). A similar hypothesis could not be excluded for pipefish but needs further examination.

The study of male pregnancy and genetic mating system of *Syngnathus abaster* and *Syngnathus typhle* also enlightened aspects of the reproductive biology and ethology of the two studied species (Chapter 5). The actual process of the parturition indicated the effort of both species' males to maximize the chances of their offspring survival, a strategy that is common among species exhibiting parental care (e.g. Johannes 1978; Helfman 1993; Eldreidge et al. 1991, Fujita and Kohda 1998). However, in order to test this theory the actual process of parturition should be studied in a natural population were the animals are free from any artificial constrains or stress that might deduce labor.

One of the advantages of male pregnancy for the two species is that their offspring are fully formed individuals (Silva et al. 2006b; Sommer et al. 2012). In the present thesis this resemblance was shown at 1st day newborn individuals by behavioral (distribution in the water column), qualitative observations (coloration) morphometric and meristic (dorsal fin rays, body rings) characters. The resemblance of the newborn individuals to the adults was so strong that discrimination between juveniles of the two species was possible from the first day that they were born. An additional step to the results of the present thesis would be to compare the growth of juveniles of both species under reared and/or natural conditions and examine any possible interspecies differences that may appear until they reach maturation.

The genetic mating system of *S. abaster* confirmed the species polygynandrous behavior with a higher number of females donors revealed so far. At the same time *S. typhle* was characterized as polygynous. However as already discussed, this outcome needs to be further investigated due to the low number of the examined broods. The study of both species genetic mating system was based on reconstruction of maternal genotypes (Colony software, Jones and Wang 2009). Despite the high possibility of correct reconstruction of the maternal genotype and assignment of offspring genotype to these mothers, it would be interesting to validate these results with field observations and/or by genotyping a larger female-wild population sample in order to assign offspring directly to their female donors. By the direct assignment there would be no doubt on the maternal genotype and thus the intensity of polygamy will be brought into proportion without being under or over estimated.

Phylogenetic and morphometric relationship studies (Chapters 3, 4) showed that *S. abaster* and *S. typhle* species, even though congeneric and sympatric along the coastline of Greece, are genetically and morphologically distinct. No intermediate haplotypes or morphotypes were found between the two species, indicating lack of contemporary hybridization[5]. However a different pattern of intraspecies differentiation was observed revealing the effect of past and more contemporary drives.

In particular, molecular analysis (Chapter 3) revealed that *S. abaster* exhibited a deep genetic structuring along the Greek coastline forming distinct clades between Ionian and Aegean Seas. At the same time, *S. typhle* exhibited a shallow (even non- existent) genetic structure which was uncorrelated to geographical boundaries. As already discussed (Chapter III) it seems, that during the Pleistocene period – and most likely the LGM- both species abandoned the Greek coastline and retreated in protected refugia. However, it is most likely that these refugia differed spatially and numerically. The populations of *S. abaster* that postglacially recolonized the Greek coastline most likely originated from at least two refugia i.e. one for the Aegean and one for the Ionian Sea populations. Contrary, contemporary Greek population of *S. typhle* most probably originated from one refugium situated in the Eastern Mediterranean Sea.

The limited dispersal ability due to the benthic behavior of both juvenile and adult specimens of *S. abaster* most probably prevented an extensive contemporary gene flow and ultimately led to the nowadays observed genetic structuring between the two Seas (Slatkin 1987; Pineda et al. 2007; Schunter et al. 2011). On the other hand, the increased potential for passive transportation of the benthopelagic *S. typhle* could have resulted in a contemporary gene flow even between remote populations, sustaining thus the common genetic background (Slatkin 1987; Pineda et al. 2007; Schunter et al. 2011).

[5] Hybridization of European *S. typhle* and *S. taenionotus* species occurred between 0.23 to 1.11 Mya (Hablutzel 2009).

Despite the differences in the genetic structure of the two species morphometric analysis indicated a similar pattern for them (Chapter 4). In particular Ionian and Aegean Sea phenotypes were distinct for both species. As already discussed, morphometric characters are under the influence of both genetic background (Griffiths et al. 2010) and natural selection/local adaptations (Arnold 1983; Lande and Arnold 1983; James 1983). Given that Ionian and Aegean Seas are characterized by different and variable environmental and physicochemical conditions (Coll et al. 2010) it is suggested that besides the level of genetic structuring the individuals of both species evolved local adaptations that added to their phenotypic profile (Corti et al. 1996; Clabaut et al. 2007).

As already mentioned seven more syngnathid species (*Hippocampus hippocampus*, *Hippocampus guttulatus*, *Nerophis ophidion*, *Syngnathus acus*, *Syngnathus phlegon*, *Syngnathus taenionutus* and *Syngnathus tenuirostris*) (Economidis and Bauchot 1976; Dawson 1986; Papakonstantinou 1988) exist along the Greek coastline. A question arising from the results of the present thesis is if the genetic and morphometric pattern of these species resembles that of *S. typhle*, *S. abaster* or follows a new motif. The study of as many as possible sister and sympatric occurring species could help to unravel the effect of past and present drives on syngnathids genetic structure and even indicate a pattern that could be generalized in other nearshore organisms, too. Also such an extensive study could define the effect of genetic structure and local adaptations on the morphology of syngnathids and indicate the most important factor that shaped it. Finally the sampling size could be wider, including individuals from the Ionian and Aegean Seas islands, and providing thus a detailed pattern of genetic and morphometric structure along the entire Greek coastline.

In conclusion, the results of the present thesis helped in the understanding of the biology, genetic mating system and phylogenetic relationship of two of the most abundant pipefishes along the Greek coastline. It provided, for the first time, data on many aspects of the above fields and set the grounds for further research to come.

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Appendix

Table 2.1. The composition of ichthyofauna of Drepano station on a bimonthly basis in the present study.

Πίνακας 2.1. Σύσταση ιχθυοπανίδας του σταθμού του Δρεπάνου σε διμηνιαία βάση κατά τη διάρκεια της παρούσας μελέτης.

Family	Genus	Species	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec
Atherinidae	*Atherina*	*A. boyeri*	2315	989	4133	1844	473	200
Belonidae	*Belone*	*B. belone*	0	0	0	1	5	1
Blenniidae	*Parablennius*	*P. gattorugine*	0	2	39	23	1	0
		P. incognitus	1	5	1	3	3	9
		P.sanguinolentus	0	0	0	71	0	1
		P. tentacularis	0	0	0	0	0	1
	Salaria	*S. pavo*	0	5	2	22	1	0
Callionymidae	*Callionymus*	*C. pusillus*	0	0	0	3	0	3
		C. risso	0	0	2	0	0	0
Clupeidae	*Sardina*	*S. pilchardus*	0	0	156	8	23	0
Engraulidae	*Engraulis*	*E. encrasicolus*	0	2	44	0	98	0
Gobidea		*Gobidea sp.*	942	782	833	832	554	21
Labridae	*Symphodus*	*S. cinereus*	29	126	12	20	15	42
		S. mediterraneus	0	0	0	0	0	1
		S. ocellatus	0	11	1	0	0	2
		S. tinca	0	0	1	4	0	0
Mugilidae	*Chelon*	*C. labrosus*	0	0	2	34	1	0
	Liza	*L. aurata*	1008	647	218	65	41	1078
		L. saliens	2	0	7	56	26	0
	Mugil	*M. cephalus*	4	0	0	0	0	0
Mullidae	*Mullus*	*M. surmuletus*	0	0	135	81	21	1
Scophthalmidae	*Scophthalmus*	*S. maximus*	0	2	0	0	0	0
Scorpaenidae	*Scorpaena*	*S. porcus*	1	2	0	4	1	3
Soleidae	*Solea*	*S. solea*	4	10	0	1	2	0
Sparidae	*Diplodus*	*D. annularis*	0	14	0	6	13	2
		D. puntazzo	4	79	8	31	1	0
		D. sargus	0	0	2	15	0	0
		Diplodus sp.	0	0	8	1	0	0
		D. vulgaris	0	35	11	1	0	0
	Sarpa	*S. salpa*	0	298	50	8	10	1
	Sparus	*S. aurata*	0	0	0	0	1	1
	Nerophis	*N. ophidion*	0	1	1	1	0	2
Syngnathidae	*Syngnathus*	*S. abaster*	16	14	62	74	129	18
		S. acus	0	6	4	6	2	0
	Syngnathus	*S. typhle*	1	23	75	62	113	13
Trachinidae	*Echiichthys*	*E. vipera*	0	0	0	1	1	0
Triglidae	*Lepidotrigla*	*L. cavillone*	0	4	1	0	0	0

Table 2.2. The composition of ichthyofauna of Neochori station on a bimonthly basis in the present study.

Πίνακας 2.2. Σύσταση ιχθυοπανίδας του σταθμού του Νεοχωρίου σε διμηνιαία βάση κατά τη διάρκεια της παρούσας μελέτης.

Family	**Genus**	**Species**	**Jan-Feb**	**Mar-Apr**	**May-Jun**	**Jul-Aug**	**Sep-Oct**	**Nov-Dec**
Anguillidae	*Anguilla*	*A. anguila*	0	0	0	1	0	0
Atherinidae	*Atherina*	*A. boyeri*	440	192	1557	595	890	1276
Belonidae	*Belone*	*B. belone*	0	5	8	5	62	2
Blenniidae	*Salaria*	*S. pavo*	0	0	19	0	6	0
Blenniidae	*Parablennius*	*S. gattorugine*	3	0	1	0	0	0
Blenniidae	*Parablennius*	*P. incognitus*	0	0	0	0	0	1
Blenniidae	*Parablennius*	*P. sanguinolentus*	0	0	1	10	0	0
Callionymidae	*Callionymus*	*C. pusillus*	1	0	0	0	0	0
Clupeidae	*Sardina*	*S. pilchardus*	0	0	0	0	1	0
Cyprinodontidae	*Aphanius*	*A. fasciatus*	0	24	6	9	1	6
Gobidea		*Gobidea sp.*	109	14	68	41	33	1
Labridae	*Symphodus*	*S. doderleini*	0	0	0	1	0	0
Labridae	*Symphodus*	*S. ocellatus*	8	0	1	0	0	0
Mugilidae	*Chelon*	*C. labrosus*	0	0	0	0	0	0
Mugilidae	*Liza*	*L. aurata*	233	323	45	26	1	68
Mugilidae	*Liza*	*L. saliens*	18	1	51	8	34	0
Mugilidae	*Mugil*	*M. chephalus*	0	0	0	0	0	2
Mullidae	*Mullus*	*M. surmuletus*	0	0	3	7	0	0
Soleidae	*Solea*	*S. solea*	1	8	2	3	0	3
Sparidae	*Diplodus*	*D. annularis*	1	0	0	0	0	0
Sparidae	*Sarpa*	*S. salpa*	0	0	16	0	0	0
Syngnathidae	*Nerophis*	*N. ophidion*	0	1	6	0	9	0
Syngnathidae	*Syngnathus*	*S. abaster*	1	23	75	62	113	13
Syngnathidae	*Syngnathus*	*S. acus*	0	0	8	14	1	0
Syngnathidae	*Syngnathus*	*S. typhle*	27	28	92	137	111	42

Table 2.3. Chi square test (x^2) values for the sex ratio of males to females and adults to unsexed specimens of *S. abaster* and *S. typhle* species from Drepano and Neochori stations in the present study (*p*, level of significance).

Πίνακας 2.3. Αποτελέσματα του τεστ x^2 για την αναλογία φύλων των αρσενικών προς θηλυκών και ενήλικων προς αδιευκρίνιστου φύλου ατόμων των ειδών *S. abaster* και *S. typhle* από τους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά τη διάρκεια της παρούσας μελέτης (*p*, επίπεδο σημαντικότητας).

Species	Station	Month	Males	Females	Adult	Unsexed	Males: Females		Adult:Unsexed	
			N	N	N	N	Chi-square test	p	Chi-square test	p
S. abaster	Drepano	Jan-Feb	9	4	13	3	1.92	>0.05	6.25	<0.05
		Mar-Apr	3	10	13	1	3.77	>0.05	10.29	<0.01
		May-Jun	19	23	42	20	0.38	>0.05	7.81	<0.01
		Jul-Aug	23	25	48	26	0.08	>0.05	6.54	<0.05
		Sep-Oct	35	75	110	19	14.55	<0.001	64.19	<0.001
		Nov-Dec	7	9	16	2	0.25	>0.05	10.89	<0.001
	Neochori	Jan-Feb	2	3	5	0	0.20	>0.05	5.00	
		Mar-Apr	2	4	6	13	0.67	>0.05	2.58	>0.05
		May-Jun	7	5	12	25	0.33	>0.05	4.57	<0.05
		Jul-Aug	25	34	59	55	1.37	>0.05	0.14	>0.05
		Sep-Oct	6	17	23	8	5.26	<0.001	7.26	<0.01
		Nov-Dec	0	2	2	3	2.00	>0.05		
S. typhle	Drepano	Jan-Feb	1	0	1	0				
		Mar-Apr	7	15	22	1	2.91	>0.05	19.17	<0.001
		May-Jun	32	34	66	9	0.06	>0.05	43.32	<0.001
		Jul-Aug	25	31	56	6	0.64	>0.05	40.32	<0.001
		Sep-Oct	41	47	88	25	0.41	>0.05	35.12	<0.001
		Nov-Dec	7	5	12	1	0.33	>0.05	9.31	<0.01
	Neochori	Jan-Feb	12	15	27	0	0.33	>0.05		
		Mar-Apr	15	11	26	2	0.62	>0.05	20.57	<0.001
		May-Jun	40	16	56	36	10.29	<0.01	4.35	<0.05
		Jul-Aug	45	50	95	42	0.26	>0.05	20.50	<0.001
		Sep-Oct	28	52	80	31	7.20	<0.01	21.63	<0.001
		Nov-Dec	11	28	39	3	7.41	<0.01	30.86	<0.001

Table 2.4. Chi square test (x^2) values for the ratio of brooding to non-brooding males and non-brooding to female individuals of *S. abaster* and *S. typhle* species from the stations of Drepano and Neochori during the breeding period of the present study (*p*, level of significance).

Πίνακας 2.4. Αποτελέσματα του τεστ x^2 για την αναλογία κυοφορούντων ως προς μη-κυοφορούντων αρσενικών ατόμων και κυοφορούντων αρσενικών ως προς θηλυκών ατόμων των ειδών *S. abaster* και *S. typhle* από τους σταθμούς του Δρεπάνου και του Νεοχωρίου κατά την αναπαραγωγική περίοδο της παρούσας μελέτης (*p*, επίπεδο σημαντικότητας).

Species	Station	Month	Males		Females	Non-brooding:Brooding males		Non-brooding males:Females	
			Non-brooding	Brooding		Chi-square	P	Chi-square	P
			N	N	N				
S. abaster	Drepano	May-Jun	5	14	23	4.26	<0.05	11.57	<0.05
		Jul-Aug	10	13	25	0.39	>0.05	6.43	<0.05
		Sep-Oct	24	11	75	4.83	<0.05	26.27	<0.001
	Neochori	May-Jun	1	6	5	3.57	>0.05	2.67	>0.05
		Jul-Aug	11	14	34	0.36	>0.05	11.76	<0.001
		Sep-Oct	3	3	17	0.00	>0.05	9.80	<0.001
S. typhle	Drepano	Mar-Apr	3	4	15	0.14	>0.05	8.00	<0.01
		May-Jun	15	17	34	0.13	>0.05	7.37	<0.01
		Jul-Aug	10	15	31	1.00	>0.05	10.76	<0.01
		Sep-Oct	14	27	47	4.12	<0.05	17.85	<0.001
	Neochori	Mar-Apr	9	6	11	0.60	>0.05	0.20	>0.05
		May-Jun	22	18	16	0.40	>0.05	0.95	>0.05
		Jul-Aug	17	28	50	2.69	>0.05	16.25	<0.001
		Sep-Oct	21	7	52	7.00	<0.01	13.16	<0.001

Table 2.5. Total length (TL mm) range of males and females of a) *S. abaster* and b) *S. typhle* species caught in Drepano and Neochori stations used in the estimation of the logistic regression curves for the estimation of the L_{50} in present study (*Min*, minimum total length; *Max*, maximum total length)

Πίνακας 2.5. Εύρος το ολικού μήκους (TL mm) των αρσενικών ατόμων και θηλυκών ατόμων των ειδών a) *S. abaster* και b) *S. typhle* από τους σταθμούς του Δρεπάνου και του Νεοχωρίου Καμπύλη τα οποία χρησιμοποιήθηκαν στον υπολογισμό της λογιστικής παλινδρόμησης για την εκτίμηση του L_{50} στην παρούσα μελέτη study (*Min*, ελάχιστο ολικό μήκος; *Max*, μέγιστο ολικό μήκος)

a)		**Males**		**Females**	
Station	**Month**	**Min**	**Max**	**Min**	**Max**
Drepano	Jan-Feb	95.0	95.0		
	Mar-Apr	88.0	172.0	121.0	243.0
	May-Jun	82.0	237.0	84.0	193.0
	Jul-Aug	60.5	214.0	80.4	194.0
	Sep-Oct	60.0	214.0	80.0	220.0
	Nov-Dec	105.0	155.0	90.0	207.0
	Total	73.0	237.0	80.0	243.0
Neochori	Jan-Feb	101.0	157.0	102.0	181.0
	Mar-Apr	77.0	185.8	98.7	151.1
	May-Jun	67.0	163.0	78.0	166.0
	Jul-Aug	60.1	187.0	78.0	171.0
	Sep-Oct	60.7	172.0	74.0	182.0
	Nov-Dec	86.0	188.0	77.0	183.0
	Total	67.0	188.0	74.0	183.0

b)		**Males**		**Females**	
Station	**Month**	**Min**	**Max**	**Min**	**Max**
Drepano	Jan-Feb	95.0	95.0		
	Mar-Apr	88.0	172.0	121.0	243.0
	May-Jun	59.8	237.0	84.0	193.0
	Jul-Aug	104.0	214.0	80.4	194.0
	Sep-Oct	61.8	214.0	80.0	220.0
	Nov-Dec	105.0	155.0	90.0	207.0
	Total	73.0	237.0	80.0	243.0
Neochori	Jan-Feb	101.0	157.0	102.0	181.0
	Mar-Apr	77.0	185.8	98.7	151.1
	May-Jun	60.6	163.0	63.1	166.0
	Jul-Aug	61.2	187.0	60.2	171.0
	Sep-Oct	60.5	172.0	74.0	182.0
	Nov-Dec	86.0	188.0	77.0	183.0
	Total	67.0	188.0	74.0	183.0

Table 3.1. Number of individuals of *S. abaster* and *S. typhle* species collected from the 19 sampling stations along the mainland sublittoral coastline of Greece
Πίνακας 3.1. Αριθμός ατόμων των ειδών *S. abaster* και *S. typhle* τα οποία συλλέχθησαν από 19 σταθμούς δειγματοληψίας κατά μήκος της παράκτιας παραλιακής ζώνης της ηπειρωτικής Ελλάδας

Sea	Geographical region	Sampling station	Species	
			S. abaster	***S. typhle***
			N	**N**
Ionian Sea	Port of Igoumenitsa	Drepano	10	6
	Amvrakikos Gulf	Neochori	7	7
	Mitikas	Mitikas	11	3
	Tourlida	Tourlida	6	0
		Kalogria	13	0
		Katakolo	3	2
	Peloponnese	Kotichi	7	0
		Kaiafa	5	0
		Gialova	0	1
Aegean Sea	Peloponnese	Moustos	5	0
	Korinthiakos Gulf	Kechries	5	1
	Evoikos Gulf	Livanata	3	3
		Karavomilos	10	0
	Pagasitikos Gulf	Lechonia	5	0
	Thermaikos Gulf	Korinnos	15	5
		Pilaia	8	5
	Chalkidiki	Vourvourou	4	3
		Porto Koufo	8	0
	Thrace	Vassova	7	0
		Drana	10	2

Table 3.2.a. Haplotype designation and variable nucleotide positions among 78 haplotypes revealed after analysis of the mitochondrial Control Region of *S. abaster*. Numbers refer to underlined positions in Figure 3.6. For every haplotype nucleotides are given when they are different from the consensus sequence, while identity is shown by dots.

Πίνακας 3.2.a. Διαφορετικοί απλότυποι και πολυμορφικές θέσεις μεταξύ των 78 απλοτύπων που προέκυψαν από την ανάλυση της περιοχής ελέγχου του μιτοχονδριακού DNA του είδους *S. abaster*. Οι αριθμοί αντιστοιχούν στις υπογραμμισμένες θέσεις στην Εικόνα 3.6. Τα νουκλεοτίδια κάθε πολυμορφικής θέσης δίνονται όταν αυτά διαφέρουν από την αλληλουχία αναφοράς, ενώ η ομοιότητα συμβολίζεται με τελείες.

	15	28	33	41	45	68	74	85	89	100	102	112	120	135	145	163	240	280	393	416	428	429	440	458
1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	G	.	.	T	.	.	C	.
3	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	C	.
4	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.
5	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	G	T	.	.	.	.	.	.
6	A	.	.	.	.	.	.	.	.	.	.	.	.	G	.	T	G	T	.	.	.	.	.	.
7	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	G	T	.	.	.	.	.	.
8	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	G	T	.	.	.	.	.	.
9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	C	.
13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
14	.	.	.	.	.	A	.	.	.	.	.	.	.	G	.	T	.	.	.	.	T	C	.	.
15	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	C	.
16	.	.	.	.	.	A	.	.	.	.	.	.	.	G	.	.	G	.	.	.	T	C	.	.
17	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.
18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.
19	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
20	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	G	.	.	.	T	C	.	.
21	.	.	T	G	A	G	.	C	.	.	T	G	.	G	.	T	.	.	.	.	.	.	.	T
22	.	.	T	G	A	G	.	C	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	T
23	.	.	T	G	A	G	.	C	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	T
24	.	.	T	G	A	G	.	C	.	.	T	G	G	G	.	T	.	.	A	.	.	.	.	T
25	.	.	T	G	A	G	.	C	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	T
26	.	.	T	G	G	G	.	.	C	.	.	.	.	G	.	T	.	.	.	.	.	.	.	T
27	.	.	T	G	A	G	.	C	.	.	T	G	.	.	.	T	.	.	.	.	.	.	.	T
28	.	.	T	G	G	G	.	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T
29	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
30	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	C	.	.
31	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	C	.	.
32	.	.	T	G	G	.	.	A	C	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.
33	.	.	T	G	G	.	.	A	C	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.
34	.	G	T	G	G	.	.	A	C	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.
35	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
36	.	.	T	G	G	.	.	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.

Table 3.2.a. Continued
Πίνακας 3.2.a. Συνέχεια

	462	498	584	585	601	616	629	631	633	640	641	642	643	673	676	677	678	679	680	690	691	693	695
1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.
6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.
7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	C	.	.
8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	T	.	.	.	.	C	.	.
9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
12	C	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
17	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
18	C	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.
19	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
21	.	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
22	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	T
23	.	.	.	.	.	.	.	.	.	.	.	G	C	C	C	.	.	.	.	.	.	C	T
24	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	T
25	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	T
26	.	.	.	.	T	T	.	T	.	T	.	G	.	.	C	.	.	.	.	.	.	.	T
27	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	T
28	C	.	.	.	.	.	.	T	.	T	.	G	.	.	C	.	.	.	.	.	.	.	T
29	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
30	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
31	C	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
32	.	.	T	A	.	.	.	.	A	T	.	G	.	.	C	.	.	.	G	.	.	.	.
33	.	.	T	A	.	.	.	.	A	T	.	G	.	.	C	.	.	.	G	.	.	.	.
34	.	.	T	A	.	.	.	.	A	T	.	G	.	.	C	.	.	.	G	.	.	.	.
35	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	T
36	.	.	.	.	.	.	T	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	.

Table 3.2.a. Continued
Πίνακας 3.2.a. Συνέχεια

	725	732	734	735	737	739	750	752	756	761	762	763	764	765	766	770	773	778	790	795	801	802	804
1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.
3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
6	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
7	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.
8	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.
9	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	C	.
14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
17	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
19	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
21	.	.	.	.	C	.	C	.	C	.	.	.	.	.	.	G	.	.	.	.	A	C	.
22	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
23	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
24	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
25	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.
26	.	.	.	.	.	.	C	T	.	.	T	A	.	A	.	.	.	.	.	.	A	C	.
27	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
28	.	.	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.
29	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
30	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
31	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
32	.	.	.	.	C	.	C	.	.	.	.	.	.	.	T	.	.	.	.	.	A	C	.
33	.	.	.	.	C	.	C	.	.	.	.	.	.	.	T	.	.	.	T	.	A	C	.
34	.	.	.	.	C	.	C	.	.	.	.	.	.	.	T	.	.	.	.	.	A	C	.
35	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
36	.	G	.	.	C	A	C	T	.	.	.	.	.	.	.	.	.	.	.	.	A	C	T

Table 3.2.a. Continued. **Πίνακας 3.2.a.** Συνέχεια

	15	28	33	41	45	68	74	85	89	100	102	112	120	135	145	163	240	280	393	416	428	429	440	458
37	.	.	T	G	G	.	.	.	C	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.
38	.	.	T	G	A	G	.	C	.	.	T	G	.	.	.	T	.	.	.	.	.	.	.	T
39	.	.	T	G	A	G	.	C	.	A	T	.	.	G	.	T	.	.	A	.	.	.	.	T
40	.	.	T	G	G	.	.	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
41	.	.	T	G	A	G	.	C	.	A	T	.	.	G	.	T	.	.	.	.	.	.	.	T
42	.	.	T	G	G	.	.	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
43	.	.	T	G	A	G	.	C	.	A	T	.	.	G	.	T	.	.	.	.	.	.	.	T
44	.	.	T	G	A	G	.	C	.	.	T	G	.	.	.	T	.	.	.	.	.	.	.	T
45	.	.	T	G	G	.	.	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
46	.	.	T	G	A	G	.	C	.	A	T	.	.	G	.	T	.	.	.	.	.	.	.	T
47	.	.	T	G	G	G	.	A	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T
48	.	.	T	G	A	G	.	C	.	.	T	G	.	.	.	T	.	.	.	.	.	.	.	T
49	.	.	T	G	A	G	.	C	.	.	T	G	.	.	.	T	.	.	.	.	.	.	.	T
50	.	.	T	G	A	G	.	C	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	T
51	.	.	T	G	A	G	.	C	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	T
52	.	.	T	G	G	G	.	C	.	.	T	G	.	G	.	T	.	.	.	.	.	.	.	T
53	.	.	T	G	A	G	.	C	.	.	T	.	.	.	.	T	.	.	A	.	.	.	.	T
54	.	.	T	G	A	G	.	C	.	.	T	G	.	.	.	T	.	.	A	.	.	.	.	T
55	.	.	T	G	A	G	.	C	.	.	T	G	.	.	.	T	.	.	.	.	.	.	.	T
56	.	.	T	G	A	G	.	C	.	A	T	G	G	.	.	T	.	.	.	.	.	.	.	T
57	.	.	T	G	A	G	.	C	.	.	T	G	.	.	.	T	.	.	.	.	.	.	.	T
58	.	.	T	G	A	G	.	C	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	T
59	.	.	T	G	G	G	.	C	.	.	T	G	.	G	.	T	.	.	.	.	.	.	.	T
60	.	.	T	G	A	G	.	C	.	.	T	G	.	.	.	T	.	.	.	.	.	.	.	T
61	.	.	T	G	A	G	.	C	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	T
62	.	.	T	G	A	G	.	C	.	.	T	G	.	.	.	T	.	.	.	.	.	.	.	T
63	.	G	T	G	A	G	.	C	.	.	T	G	.	.	.	T	.	.	A	.	.	.	.	T
64	.	.	T	G	G	G	.	C	.	.	T	G	.	.	.	T	.	.	.	.	.	.	.	T
65	.	.	T	G	A	G	.	.	.	A	T	G	.	.	.	T	.	.	.	.	.	.	.	T
66	.	.	T	G	A	G	.	C	.	.	T	G	.	.	.	T	.	.	.	.	.	.	.	T
67	.	.	T	G	G	G	.	.	.	A	T	G	.	G	.	T	.	.	.	.	.	.	.	T
68	.	.	T	G	A	G	.	T	.	.	T	G	.	.	.	T	.	.	.	.	.	.	.	T
69	.	.	T	G	G	.	.	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
70	.	.	T	G	G	.	.	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
71	.	.	T	G	G	.	.	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
72	.	.	T	G	G	.	.	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
73	.	.	T	G	A	G	.	C	.	.	T	G	.	.	.	T	.	.	.	.	.	.	.	T
74	.	.	T	G	G	G	T	A	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T
75	.	.	T	G	A	G	.	C	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	T
76	.	.	T	.	A	G	.	C	.	.	T	G	.	.	.	T	.	.	.	.	.	.	.	T
77	.	.	T	G	A	G	.	T	.	.	T	G	.	G	.	T	.	.	.	.	.	.	.	T
78	.	.	T	G	A	G	.	C	.	.	T	G	.	.	.	T	.	.	.	.	.	.	.	T

Table 3.2.a. Continued. **Πίνακας 3.2.a.** Συνέχεια

	462	498	584	585	601	616	629	631	633	640	641	642	643	673	676	677	678	679	680	690	691	693	695
37	.	.	.	.	.	.	T	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	.
38	.	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
39	.	.	.	.	.	.	.	.	.	.	.	G	C	C	C	.	.	.	.	.	.	C	T
40	C	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	.
41	.	.	.	.	.	.	.	.	.	.	.	G	C	C	C	.	.	.	.	.	.	C	T
42	.	.	.	.	.	.	.	.	A	.	.	G	.	.	C	.	.	.	.	.	.	.	.
43	.	.	.	.	.	.	.	.	.	.	.	G	C	C	C	.	.	.	.	.	.	C	T
44	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	T
45	.	.	.	.	.	.	.	.	A	.	.	G	.	.	C	.	.	.	.	.	.	.	.
46	C	.	.	.	.	.	.	.	.	.	.	G	C	C	C	.	.	.	.	.	.	C	T
47	C	.	.	.	.	.	.	T	.	T	.	G	.	.	C	.	T	.	.	.	.	.	T
48	.	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
49	.	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
50	.	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
51	.	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
52	C	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
53	.	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
54	.	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
55	C	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	A	.	.	.	.	.	T
56	.	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
57	.	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
58	C	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	T
59	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	T
60	C	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
61	C	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
62	.	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	G	.	C	T
63	.	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
64	C	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
65	.	.	.	.	.	.	.	.	.	.	T	G	C	.	C	.	.	.	.	.	.	C	T
66	C	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	.	T
67	.	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
68	.	C	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
69	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	.
70	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	.
71	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	.
72	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	.
73	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	T
74	C	.	.	.	T	.	.	T	.	T	.	G	.	.	C	.	.	.	.	.	.	.	T
75	.	.	.	.	.	T	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	T
76	C	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	T
77	.	.	.	.	.	.	.	.	.	.	.	G	C	.	C	.	.	.	.	.	.	C	T
78	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	T

Table 3.2.a. Continued. **Πίνακας 3.2.a.** Συνέχεια

	725	732	734	735	737	739	750	752	756	761	762	763	764	765	766	770	773	778	790	795	801	802	804
37	.	G	.	.	C	A	C	T	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.
38	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
39	.	.	.	A	C	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
40	.	.	.	.	C	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
41	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
42	.	.	.	.	C	.	C	T	.	.	.	.	.	.	.	.	A	.	.	.	A	C	.
43	.	.	.	.	C	.	C	.	.	.	.	.	C	.	.	G	.	.	.	.	A	C	.
44	.	.	.	.	C	.	C	.	.	A	.	.	.	.	.	G	.	.	.	.	A	C	.
45	.	.	.	.	C	.	C	T	.	.	.	.	.	.	.	.	A	G	.	.	A	C	.
46	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
47	.	.	.	.	C	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.
48	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
49	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
50	.	.	.	.	C	.	C	.	.	.	T	.	.	.	.	G	.	.	.	.	A	C	.
51	.	.	.	.	C	.	C	.	.	.	T	.	.	C	.	G	.	.	.	.	A	C	.
52	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
53	.	.	.	.	C	.	C	.	.	.	T	.	.	.	.	G	.	.	.	.	A	C	.
54	.	.	.	.	C	A	.	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
55	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
56	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
57	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	G	.	.	T	.	A	C	.
58	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
59	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
60	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
61	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
62	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
63	.	.	.	.	C	A	.	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
64	.	.	A	.	C	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
65	.	.	.	.	C	.	C	.	.	.	.	T	.	.	.	G	.	.	.	.	A	C	.
66	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	T	C	.
67	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
68	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	A	C	.
69	.	.	.	.	C	.	C	T	.	.	.	.	.	.	.	.	.	G	.	.	A	C	.
70	.	.	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.
71	.	.	.	.	C	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.
72	.	.	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	G	.	.	A	C	.
73	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	G	A	C	.
74	.	.	.	.	C	.	C	T	.	.	T	T	.	.	.	.	.	.	.	.	A	C	.
75	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
76	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
77	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
78	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.

Table 3.2.b. Haplotype designation and variable nucleotide positions among 14 haplotypes revealed after analysis of nuclear Locus A1 of *S. abaster*. Numbers refer to underlined positions in Figure 3.10. For every haplotype nucleotides are given when they are different from the consensus sequence, while identity is shown by dots.
Πίνακας 3.2.b. Διαφορετικοί απλότυποι και πολυμορφικές θέσεις μεταξύ των 14 απλοτύπων που προέκυψαν από την ανάλυση του πυρηνικού τόπου A1 του είδους *S. abaster*. Οι αριθμοί αντιστοιχούν στις υπογραμμισμένες θέσεις στην Εικόνα 3.10. Τα νουκλεοτίδια κάθε πολυμορφικής θέσης δίνονται όταν αυτά διαφέρουν από την αλληλουχία αναφοράς, ενώ η ομοιότητα συμβολίζεται με τελείες.

	3	**32**	**45**	**65**	**77**	**106**	**121**	**163**	**180**	**195**	**196**	**206**	**219**	**234**	**238**	**244**	**245**	**298**	**386**	**397**	**425**
1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
3	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
5	.	G	.	.	.	.	.	.	.	C	C	.	A	G	.	T	G	T	.	.	.
6	.	G	.	.	.	.	A	.	.	C	C	.	.	G	.	T	G	T	.	.	.
7	.	G	.	T	.	.	A	.	.	C	C	.	.	G	.	T	G	T	.	.	.
8	A	G	.	.	.	.	.	T	.	.	.	.	.	G	.	T	G	T	.	.	.
9	A	G	.	.	T	.	.	.	T	.	.	.	.	G	.	T	G	T	.	.	.
10	.	G	.	.	.	A	A	.	.	C	C	.	.	G	.	T	G	T	.	.	.
11	.	G	.	.	.	.	A	.	.	C	C	.	.	G	.	T	G	T	A	.	.
12	.	G	.	.	.	.	.	.	.	C	C	.	A	G	.	T	G	T	.	C	.
13	.	G	.	.	.	.	A	.	.	C	C	.	.	G	.	T	G	T	.	G	.
14	.	G	.	.	.	.	A	.	.	C	C	G	.	G	.	T	G	T	.	.	.

Table 3.2.c. Haplotype designation and variable nucleotide positions among 17 haplotypes revealed after analysis of the mitochondrial Control Region of *S. typhle*. Numbers refer to underlined positions in Figure 3.14. For every haplotype nucleotides are given when they are different from the consensus sequence, while identity is shown by dots.

Πίνακας 3.2.c. Διαφορετικοί απλότυποι και πολυμορφικές θέσεις μεταξύ των 14 απλοτύπων που προέκυψαν από την ανάλυση της περιοχής ελέγχου του μιτοχονδριακού DNA του είδους *S. typhle*. Οι αριθμοί αντιστοιχούν στις υπογραμμισμένες θέσεις στην Εικόνα 3.14. Τα νουκλεοτίδια κάθε πολυμορφικής θέσης δίνονται όταν αυτά διαφέρουν από την αλληλουχία αναφοράς, ενώ η ομοιότητα συμβολίζεται με τελείες.

	5	**41**	**43**	**89**	**100**	**102**	**112**	**119**	**134**	**163**	**233**	**240**	**440**	**460**	**462**	**468**	**490**	**632**	**671**	**720**	**435**	**801**
1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	.	.	.	.	G	.	G	G	.	.	.	.	C	A	.	.	.	.	.	.	C	.
3	.	G	.	.	G	.	G	.	.	.	.	.	C	A	.	.	.	.	.	.	C	.
4	.	.	.	.	G	C	.	.	.	.	G	.	.	.	.	.	A	.	G	G	C	.
5	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	.	.	.	.	.	.	.
6	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
7	.	.	.	T	G	C	G	G	.	.	.	.	.	.	C	.	.	.	.	.	C	.
8	.	.	.	.	.	.	G	.	G	.	.	.	C	.	.	.	.	.	.	.	.	.
9	.	.	.	T	G	C	G	G	.	.	.	G	.	.	C	.	.	.	.	.	C	.
10	.	.	.	.	G	C	.	.	.	.	G	G	.	.	.	.	A	.	.	G	C	.
11	C	.	.	.	G	.	G	.	.	.	.	.	C	.	.	G	.	.	.	G	C	.
12	.	G	.	.	.	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	C	C
13	.	.	.	.	.	.	G	.	G	.	.	.	C	.	.	.	.	A	.	G	C	.
14	.	G	.	T	G	.	G	.	.	.	.	G	C	A	.	.	.	.	.	G	C	.
15	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
16	.	.	.	.	.	C	G	G	G	.	.	.	C	.	.	.	.	.	.	.	C	.
17	.	G	.	T	G	.	G	.	.	.	.	.	C	A	.	.	.	.	.	G	C	.

Table 3.2.d. Haplotype designation and variable nucleotide positions among 11 haplotypes revealed after analysis of nuclear Locus A1 of *S. typhle*. Numbers refer to underlined positions in Figure 3.18. For every haplotype nucleotides are given when they are different from the consensus sequence, while identity is shown by dots.
Πίνακας 3.2.d. Διαφορετικοί απλότυποι και πολυμορφικές θέσεις μεταξύ των 11 απλοτύπων που προέκυψαν από την ανάλυση του πυρηνικού τόπου A1 του είδους *S. typhle*. Οι αριθμοί αντιστοιχούν στις υπογραμμισμένες θέσεις στην Εικόνα 3.18. Τα νουκλεοτίδια κάθε πολυμορφικής θέσης δίνονται όταν αυτά διαφέρουν από την αλληλουχία αναφοράς, ενώ η ομοιότητα συμβολίζεται με τελείες.

	30	**59**	**88**	**225**	**285**	**312**	**332**	**395**	**396**	**399**
1	.	.	.	.	.	.	.	.	.	.
2	.	.	.	.	.	.	.	A	C	.
3	.	.	.	A	.	.	.	.	.	.
4	.	.	.	.	.	.	T	.	.	.
5	C	.	.	A	.	.	.	.	.	C
6	.	.	.	.	C	.	.	.	C	.
7	.	.	.	.	.	.	.	.	C	.
8	.	.	.	.	.	T	.	.	.	.
9	C	G	.	.	.	.	.	.	.	.
10	C	G	C	.	.	.	.	.	.	.
11	.	.	C	.	.	.	.	.	.	.

Table 3.3.a. Distribution of 78 mitochondrial haplotypes among 19 *S. abaster* sampling stations (*n*, number of individuals carrying each haplotype; *Hnp*, number of copies of each haplotype in the population).
Πίνακας 3.3.a. Κατανομή των 78 απλοτύπων που ανιχνεύθηκαν κατά την ανάλυση της περιοχής ελέγχου του μιτοχονδριακού DNA του είδους *S. abaster* στους 19 σταθμούς δειγματοληψίας (*n*, ο αριθμός των ατόμων τα οποία φέρουν τον κάθε απλότυπο; *Hnp*, αριθμός αντιγράφων κάθε απλοτύπου του πληθυσμού)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**
1.Drepano	5	1	1	1																						
2. Neochori					1	1	1	1																		
3. Mitikas									1	6	1	1	1													
4.Tourlida	1													1	1	1	1	1								
5.Kalogria																			1	1	2	1	1	1	1	1
6.Katakolo			2																							
7.Kotichi																			2							
8.Kaiafa																										
9.Moustos																										
10.Kechries																										
11.Livanata																										
12.Karavomilos																										
13.Lechonia																										
14.Korinnos																										
15.Pilaia																										
16.Vourvourou																										
17.Porto Koufo																										
18.Vassova																					1	1				
19.Drana																						1				
n	6	1	3	1	1	1	1	1	1	6	1	1	1	1	1	1	1	1	3	1	3	3	1	1	1	1
Hnp	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	2	3	1	1	1	1
Haplotype frequency	2.30	1.15	2.30	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	2.30	1.15	2.30	3.45	1.15	1.15	1.15	1.15

Table 3.3.a. Continued
Πίνακας 3.3.a. Συνέχεια

	27	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**
1.Drepano																										
2. Neochori																										
3. Mitikas																										
4.Tourlida																										
5.Kalogria	1	1																								
6.Katakolo			1																							
7.Kotichi																										
8.Kaiafa				3	2																					
9.Moustos						3	1	1																		
10.Kechries									1	2	1															
11.Livanata												2	1													
12.Karavomilos														1	1	1	1	1	1	1						
13.Lechonia																					3	1	1			
14.Korinnos																								1	1	1
15.Pilaia																										
16.Vourvourou												1														
17.Porto Koufo																										
18.Vassova		1																								
19.Drana	1																									
n	2	2	1	3	2	3	1	1	1	2	1	3	1	1	1	1	1	1	1	1	3	1	1	1	1	1
Hnp	2	2	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Haplotype frequency	2.30	2.30	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	2.30	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15

Table 3.3.a. Continued
Πίνακας 3.3.a. Συνέχεια

	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78
1.Drepano																										
2. Neochori																										
3. Mitikas																										
4.Tourlida																										
5.Kalogria																										
6.Katakolo																										
7.Kotichi																										
8.Kaiafa																										
9.Moustos																										
10.Kechries																										
11.Livanata																										
12.Karavomilos																										
13.Lechonia																										
14.Korinnos	1	1	1	1	1	1	1	1																		
15.Pilaia									2	1	1	1	1													
16.Vourvourou														2	1											
17.Porto Koufo																1	4	1	1	1						
18.Vassova																					1	1	1			
19.Drana																								1	2	1
n	1	1	1	1	1	1	1	1	2	1	1	1	1	2	1	1	4	1	1	1	1	1	1	1	2	1
Hnp	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Haplotype frequency	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15

Table 3.3.b. Distribution of 14 nuclear A1 haplotypes among 17 *S. abaster* sampling stations (*n*, number of individuals carrying each haplotype; *Hnp*, number of copies of each haplotype in the population).
Πίνακας 3.3.b. Κατανομή των 14 απλοτύπων που ανιχνεύθηκαν κατά την ανάλυση του πυρηνικού τόπου A1 του είδους *S. abaster* στους 17 σταθμούς δειγματοληψίας (*n*, ο αριθμός των ατόμων τα οποία φέρουν τον κάθε απλότυπο; *Hnp*, αριθμός αντιγράφων κάθε απλοτύπου στον υπό εξέταση πληθυσμό).

	Hap1	**Hap2**	**Hap3**	**Hap4**	**Hap5**	**Hap6**	**Hap7**	**Hap8**	**Hap9**	**Hap10**	**Hap11**	**Hap12**	**Hap13**	**Hap14**
Drepano	7	1												
Neochori	4	1	1											
Mitikas	2													
Tourlida	3			1										
Kalogria	2				2	6								
Kaiafa	1			3										
Moustos							6							
Kechries							2							
Livanata								4	2					
Karavomilos					3	1								
Lechonia						3				2	1			
Korinnos					2	1					1			
Pilaia					2	3								
Vourvourou						5					3			
Porto Koufo					1	1						3	1	
Vassova						6								
Drana					1	4								1
n	19	2	1	4	11	30	8	4	2	2	5	3	1	1
Hnp	6	2	1	2	6	9	2	1	1	1	3	1	1	1
Haplotype frequency	16.22	5.41	2.70	5.41	16.22	24.32	5.41	2.70	2.70	2.70	8.11	2.70	2.70	2.70

Table 3.3.c. Distribution of 17 mitochondrial haplotypes among 10 *S. typhle* sampling stations (*n*, number of individuals carrying each haplotype; *Hnp*, number of copies of each haplotype in the population).
Πίνακας 3.3. c. Κατανομή των 17 απλοτύπων που ανιχνεύθηκαν κατά την ανάλυση της περιοχής ελέγχου του μιτοχονδριακού DNA του είδους *S. typhle* στους 10 σταθμούς δειγματοληψίας (*n*, ο αριθμός των ατόμων τα οποία φέρουν τον κάθε απλότυπο; *Hnp*, αριθμός αντιγράφων κάθε απλοτύπου στον υπό εξέταση πληθυσμό).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
1. Drepano	1	1	1	1													
2. Neochori					2	1											
3.Mitikas							2										
4.Katakolo								1									
5.Gialova									1								
6.Livanata										2	1						
7.Korinnos							2					1					
8.Pilaia												1	1	1			
9.Vourvourou							1								1	1	
10.Vassova																	1
n	1	1	1	1	2	1	5	1	1	2	1	2	1	1	1	1	1
Hnp	1	1	1	1	1	1	3	1	1	1	1	2	1	1	1	1	1
Haplotype frequency	5.00	5.00	5.00	5.00	5.00	5.00	15.00	5.00	5.00	5.00	5.00	10.00	5.00	5.00	5.00	5.00	5.00

Table 3.3.d. Distribution of 11 nuclear A1 haplotypes among 11 *S. typhle* sampling stations (*n*, number of individuals carrying each haplotype; *Hnp*, number of copies of each haplotype in the population).
Πίνακας 3.3.d. Κατανομή των 11 απλοτύπων που ανιχνεύθηκαν κατά την ανάλυση του πυρηνικού τόπου A1 του είδους *S. typhle* στους 11 σταθμούς δειγματοληψίας. (*n*, ο αριθμός των ατόμων τα οποία φέρουν τον κάθε απλότυπο; *Hnp*, αριθμός αντιγράφων κάθε απλοτύπου στον υπό εξέταση πληθυσμό).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1.Drepano	5	1	1	1							
2.Neochori	2		1		1	2					
3.Mitikas	3		1				2				
4.Katakolo	1					1					
5.Gialova								2			
6.Kechries			1				1				
7.Livanata	5		1						1	1	
8.Korinnos	3					1					
9.Pilaia	2		1			2			1		
10.Vourvourou	6										
11.Vassova	1										1
n	9	1	6	1	1	4	2	1	2	1	1
Hp	28	1	6	1	1	6	3	2	2	1	1
Haplotype frequency	31.03	3.45	20.69	3.45	3.45	13.79	6.90	3.45	6.90	3.45	3.45

Table 5.1. Genotypes of newborn individuals of *S. abaster* species at the four studied microsatellite loci in the present study.
Πίνακας 5.1. Γενότυποι των απογόνων του είδους *S. abaster* όπως προέκυψαν από γενοτύπιση στους τέσσερις υπο μελέτη μικροδορυφορικούς τόπους.

	S.abas3		S.abas4		S.abas7		S.abas9	
samples	**Allele1**	**Allele2**	**Allele1**	**Allele2**	**Allele1**	**Allele2**	**Allele1**	**Allele2**
S.ab357	194	198	241	245	250	258	228	360
S.ab358	166	198	241	281	230	258	208	228
S.ab360	194	198	245	249	258	270		
S.ab362			241	281	238	258	208	228
S.ab363	194	198	241	245	258	270	204	228
S.ab364	166	198	241	297	230	258		
S.ab365	194	194	245	249	258	270	204	228
S.ab366	194	198	241	245	258	270	216	360
S.ab367	194	198	225	241	250	258	216	360
S.ab369	194	194	241	297	238	258	208	216
S.ab370	194	194	225	249	258	270	204	216
S.ab371	166	194	249	281	230	258	208	216
S.ab372	194	198	225	249	250	258	216	360
S.ab374	194	198	241	245	250	258	204	216
S.ab375	194	198	225	249			228	360
S.ab379	194	198	241	245	258	270	216	360
S.ab385	194	194	229	249	250	258	204	216
S.ab386	194	198	245	249	250	258	204	216
S.ab387	194	198	245	249	250	258		
S.ab388	194	198	245	249	258	270		
S.ab390	194	198			258	270	228	380
S.ab392	194	198	245	249	250	258	216	360
S.ab393	194	194	225	249	250	258	220	360
S.ab394	194	198	245	249	258	270	204	228
S.ab395	166	194	249	281	230	258	228	420
S.ab396	194	198	225	249	258	270	204	216
S.ab400	194	198	225	241	258	270		
S.ab401	194	198	245	253	258	270	228	360
S.ab403	194	194	241	297	230	258	208	228
S.ab404	194	198	225	249	258	270	228	360
S.ab415	194	194	225	241	258	270	204	216
S.ab416	194	194	241	245	250	258		
S.ab308	194	210	265	269	218	234	380	384
S.ab309	182	210	241	273	218	274	364	384
S.ab310	190	194	241	245	234	274	208	364
S.ab332	182	210	241	273	218	274	380	388
S.ab334	182	202	241	285	238	274	236	380
S.ab335	182	210	241	265	218	234	364	384
S.ab336	190	194	269	305	234	278	208	380

S.ab340	182	182	261	269	234	266	364	388
S.ab341	182	202	241	273	274	278	380	388
S.ab342	182	194	241	257	234	282	364	408
S.ab343	182	202	241	257	238	274		
S.ab345	182	190	245	269	274	278	208	364
S.ab346	190	194	241	305	234	278	208	380
S.ab347	182	194			274	274	380	388
S.ab348	174	194	245	269	234	274	364	392
S.ab349	194	202	265	269	274	278	364	388
S.ab311	182	202	269	285	274	282	236	364
S.ab313	190	194	241	245	234	278	380	388
S.ab314	182	194			234	278	364	388
S.ab315	182	194	241	245	274	278	380	388
S.ab317	194	202	241	273	274	278	364	388
S.ab318	194	198	241	261	266	274	380	392
S.ab320	194	198	257	269	234	238	364	408
S.ab321	194	202	241	257	274	282	236	380
S.ab322	182	202	257	269	238	274	236	364
S.ab323	182	198	257	269	238	274	364	408
S.ab324			241	261	234	238	380	388
S.ab325	182	202	269	285	234	282	236	380
S.ab328			269	273	234	278	380	384
S.ab329	190	194	269	285	234	238	364	408
S.ab330	182	198	269	305	234	274	208	380
S.ab331	194	210	269	273	234	278	380	384
S.ab326	190	194	241	241	234	274	380	388
S.ab423	194	198	249	253	254	262	340	372
S.ab424	194	198	253	293	250	262	228	380
S.ab425	194	198	229	361	238	266	380	380
S.ab426	202	206	237	253	246	262	340	372
S.ab430	198	202	229	293	238	266	228	380
S.ab431	198	202	249	253	238	246	228	380
S.ab434	198	202	237	253	238	246	232	380
S.ab436	194	198	229	249	254	262	340	372
S.ab437	194	198	253	361	238	250		
S.ab439	198	202	237	253	238	246	232	340
S.ab440	194	198	237	253	238	254	340	372
S.ab441	186	198	253	269	238	258	340	388
S.ab442	198	210	229	253	230	238	380	380
S.ab454	198	210	253	281	238	258	340	380
S.ab456	186	198	249	253	238	258	380	388
S.ab463	198	222	229	269	262	290		
S.ab464	194	198	229	361	250	262	212	340
S.ab465	202	206	229	281	230	262	340	340
S.ab466	198	210	253	281	230	238	208	340

S.ab469	194	198	249	253	254	262	340	388
S.ab474	198	222	253	269	238	290	380	388
S.ab476	198	210	229	245	238	238	200	340
S.ab477	186	198	229	269	238	290	340	388
S.ab478	198	202	253	253	238	258	380	380
S.ab479	186	198	245	253	238	238	380	388
S.ab480	198	202	237	253	238	246	340	372
S.ab482	186	198	241	253	238	238	200	380
S.ab486	198	222	229	269	238	290	200	380
S.ab489	198	202	229	361	238	266	228	340
S.ab490	194	198	229	249	238	254		
S.ab491	194	198	229	237	254	262	228	340
S.ab137	190	202	237	245	234	298	208	212
S.ab139	198	234	245	257	258	294	212	420
S.ab140	198	234	245	257	258	294	404	420
S.ab141	198	214	245	249	234	258	404	420
S.ab142	190	198	245	281	266	298	388	404
S.ab145	190	202	245	249	234	298	208	212
S.ab171			237	245	258	294	212	376
S.ab172	190	214	237	249	234	258	212	420
S.ab173	198	214	245	249	258	294	212	420
S.ab174	190	234	237	257			404	420
S.ab176	198	234	245	249	234	258	376	404
S.ab177	198	214	245	281	234	258	392	404
S.ab180	198	214	237	249	258	294	212	376
S.ab183	198	202	237	237	234	258	208	404
S.ab151	198	214	245	249	234	258	212	376
S.ab152	190	214	237	257	294	298	212	376
S.ab154	190	202	245	249	238	298		
S.ab157	190	214	237	281	266	298	392	404
S.ab158	198	214	237	249	258	294	212	420
S.ab159	198	234	237	249	234	258	212	420
S.ab160	190	214	237	281	234	298	212	392
S.ab161	198	234	245	257	234	258	404	420
S.ab162	198	234	237	257	234	258	404	420
S.ab153	190	214	245	257	234	298	212	420
S.ab155	190	234	245	257	234	298	212	420
S.ab163	198	202	237	249	234	298	404	420
S.ab164	198	214	237	257	258	294	376	404
S.ab165	198	218	245	249	234	258	212	420
S.ab166	198	218			234	258	404	420
S.ab167	198	202	245	249	234	298	208	212
S.ab168	190	218	237	249	238	298	404	420
S.ab169	198	202	237	237	234	258	404	420
S.ab170	198	202	245	249	234	258	212	216

S.ab186	198	222	249	305	246	270		
S.ab187	198	222	261	293	218	270	232	392
S.ab188	198	222	293	305	218	246	232	240
S.ab189	214	222	269	305	234	246	392	392
S.ab190	198	222	281	305	234	270	392	392
S.ab194	218	222	293	305	0	0	220	392
S.ab195	186	198	261	293	218	246	240	420
S.ab196	186	234	249	261	234	270	240	240
S.ab197	214	222	249	305	270	294	240	376
S.ab198	186	198	261	281	234	270	240	388
S.ab199	222	238	249	305	218	270	240	416
S.ab200	186	218	249	261	270	294	240	240
S.ab201	186	234	249	305	234	270	240	240
S.ab202	214	222	249	261	270	294	392	420
S.ab203	186	214	257	305	234	270	376	392
S.ab205	186	218	253	305	270	286	220	240
S.ab207	186	238	293	305	246	270	240	420
S.ab208	214	222	261	269	234	270	240	240
S.ab209	214	222	261	281	246	266	240	388
S.ab210	186	198	249	305	246	270	240	420
S.ab212	198	222	261	281	234	270	240	388
S.ab214	186	242	249	261	246	246	240	420
S.ab215	222	234	249	305	270	294	376	392
S.ab216	186	198	261	293	218	270	232	240
S.ab217	198	222	261	293	218	270	232	392
S.ab219	222	234	249	261	246	294	392	420
S.ab220	198	222	249	305	246	246	240	420
S.ab221	186	234	257	305	270	294	376	392
S.ab222	198	222	249	305	246	246	232	240
S.ab223	198	222	297	305	250	274		
S.ab224	186	198	249	305	218	246	220	240
S.ab39	202	206	245	265	254	278	380	388
S.ab40	178	202	225	253	206	262	400	424
S.ab41	174	222	245	253	278	278	384	388
S.ab42	174	202	245	285	230	278	384	400
S.ab43	198	222	253	265	206	226	380	400
S.ab44	174	222	253	285	270	278	364	388
S.ab45	206	222	253	277	206	246	388	424
S.ab46	174	202	253	289	270	278	384	400
S.ab47	178	202	225	253	206	262		
S.ab49	206	222	225	245	246	278	400	424
S.ab50	178	202	225	253	206	262	388	404
S.ab51	202	206	225	245	206	262	400	424
S.ab52	178	202	245	345	206	262	400	424
S.ab53	202	206	225	253	206	262	388	404

S.ab54	178	222	245	277	262	278	400	424
S.ab55	202	206	245	225	206	262	388	424
S.ab67	202	206	225	253	206	262	400	424
S.ab65			245	277	206	262	400	424
S.ab58	178	202	225	245	206	246	388	404
S.ab59	178	202	225	253	206	246	400	424
S.ab60	198	202	245	265	206	226	380	388
S.ab62	178	222	225	245	246	278	400	424
S.ab63	206	222	225	253	206	246	388	404
S.ab64	178	222	245	281	246	278	388	404
S.ab66	174	202	253	285	206	230	384	400
S.ab68	182	202	245	245	206	230	364	400
S.ab69	202	206	253	277	206	262	400	404
S.ab71	202	206	245	265	206	226	380	400
S.ab73	178	202	253	277	262	278	388	424
S.ab74	174	202	245	253	206	270	384	400
S.ab75	174	202	245	289	206	270	384	400
S.ab105			221	245	230	242	192	392
S.ab106	186	186	245	273	238	298	380	392
S.ab107	194	210	229	273	230	298	396	416
S.ab108	194	202	245	261	238	262	380	392
S.ab109	186	194	193	229	230	254	212	392
S.ab110	186	218	245	361	238	242	388	392
S.ab111	186	194	245	269	238	262	380	392
S.ab112	194	202	229	269	238	254	212	396
S.ab113	194	218	221	229	230	242	388	392
S.ab114	186	194	193	245	230	254	212	392
S.ab115	186	194	229	273	238	298	396	416
S.ab116	186	218	221	229	238	242	192	396
S.ab117	186	198	221	229	230	242	388	392
S.ab118	186	194	245	273	230	298	380	392
S.ab119	186	202	193	245	238	262		
S.ab120	194	210	229	273	238	242	392	416
S.ab98	186	202	193	245	230	262	380	396
S.ab122	186	194	229	269	230	262	380	396
S.ab123	186	202	245	269	238	262	212	392
S.ab124	186	194	193	229	230	254	212	396
S.ab125	194	198	197	245	230	262	380	396
S.ab126	194	198	245	269	238	254	380	396
S.ab127	186	186			238	242	380	392
S.ab128	194	210	237	245	238	302	380	392
S.ab130	194	210	229	273	230	242	376	392
S.ab131	186	194	197	245	238	254	380	392
S.ab99	186	186	209	245	238	298	380	392
S.ab103			221	245	238	242	380	392

S.ab132	194	218	221	229	214	238	388	396
S.ab134	186	186	229	273	230	298	392	416
S.ab136	186	198	221	229	238	242	388	392
S.ab225	198	210	249	269	218	274	380	388
S.ab226	194	198	237	241	230	254		
S.ab227	194	198	237	241	218	254	392	424
S.ab228	198	210	249	297	230	254	380	392
S.ab229	202	210	241	249	230	254		
S.ab230	198	202	249	269	218	238	376	392
S.ab231	198	210	249	269	218	242	380	388
S.ab232	198	202	209	237	230	274	376	392
S.ab234	202	210	249	269	218	274	380	388
S.ab235	198	210	249	269	218	274	380	392
S.ab236	198	202	209	249	218	274	376	388
S.ab237	202	210	249	269	218	238	380	392
S.ab239	194	202	249	297	230	254	380	392
S.ab240	198	202	209	237	230	274	380	392
S.ab241	198	210	249	269	230	274	376	392
S.ab242	202	210	249	297	218	254	380	392
S.ab243	202	210	249	297	230	282	380	388
S.ab244	194	198	249	297	230	254	380	388
S.ab245	198	198	209	237	218	238	380	392
S.ab246	198	202	241	249	230	282	388	424
S.ab247	202	210	209	249	230	238	376	388
S.ab249	198	202	237	297	230	254	388	424
S.ab250	198	202	237	249	218	254	380	392
S.ab251	202	210	249	297	230	282	380	392
S.ab252	194	198	249	297	218	282	388	424
S.ab253	198	198	237	241	218	282	380	392
S.ab254	198	202	209	249	230	238	376	392

Table 5.2. Number and genotypes of the possible mothers of *S. abaster* species as estimated from the COLONY software.
Πίνακας 5.2. Αριθμός και γενότυπος των πιθανών μητέρων του είδους *S. abaster* όπως προέκυψαν από την επεξεργασία με το πρόγραμμα COLONY

	S.abas 3			S.abas4			S.abas7			S.abas9		
Mother iD	Allele 1	Allele 2	probability	Allele 1	Allele 2	probability	Allele 1	Allele 2	probability	Allele 1	Allele 2	probability
1	194	194	0.90	225	245	1.00	250	270	1.00	204	360	1.00
2	166	194	1.00	281	297	1.00	230	238	1.00	208	420	0.97
3	198	210	0.54	237	245	0.15	270	302	0.59	380	380	0.25
3	194	210	0.41	237	249	0.14				380	392	0.10
4	202	210	1.00	265	273	1.00	218	278	1.00	384	388	1.00
5	190	194	0.70	245	305	1.00	274	278	0.96	208	388	1.00
6	198	202	1.00	257	285	1.00	238	282	1.00	236	408	1.00
7	182	198	0.62	261	261	0.14	238	266	0.99	388	392	0.99
				245	261	0.13						
				249	261	0.13						
8	174	198	0.23	245	245	0.15	234	238	0.07	380	392	0.14
	174	194	0.18	245	249	0.15	234	258	0.07	392	392	0.11
	174	202	0.14	245	253	0.08	234	274	0.07	388	392	0.10
	174	186	0.08	241	245	0.08	234	234	0.06	392	420	0.05
9	194	202	1.00	237	249	1.00	246	254	1.00	228	372	0.72
10	194	202	1.00	293	361	1.00	250	266	1.00	212	228	0.88
11	186	222	1.00	249	269	0.99	258	290	1.00	200	388	0.75
12	202	210	1.00	253	281	0.98	230	258	1.00	208	380	0.81
13	194	198	1.00	197	249	1.00	254	262	0.97	380	388	0.99
14	186	210	0.96	241	245	0.98	238	238	0.34	200	388	0.99
15	202	218	1.00	237	249	1.00	234	238	1.00	208	420	1.00
16	214	234	1.00	249	257	1.00	234	294	1.00	376	420	1.00
17	198	214	1.00	269	281	1.00	234	266	1.00	388	392	1.00
18	198	238	0.98	249	293	1.00	218	246	1.00	232	420	1.00
19	198	218	0.24	253	293	0.93	238	286	0.11	220	380	0.14
	194	218	0.18				258	286	0.10	220	392	0.11
	202	218	0.14				234	286	0.08	220	388	0.10
	186	218	0.08				230	286	0.07	220	420	0.05
20	198	198	0.24	245	297	0.15	238	274	0.08	380	392	0.03
	194	198	0.18	249	297	0.14				380	388	0.03
	198	202	0.14	253	297	0.08				388	392	0.02
	186	198	0.09	241	297	0.08				380	380	0.02
21	198	206	1.00	265	269	0.98	226	254	1.00	380	380	0.77
22	178	206	1.00	225	277	1.00	246	262	1.00	404	424	1.00
23	174	182	0.93	245	285	0.99	230	270	1.00	364	384	1.00
24	198	218	1.00	221	361	0.87	214	242	0.49	192	388	1.00

25	186	210	1.00	221	273	0.46	242	298	1.00	380	416	1.00
25				209	273	0.46			1.00			1.00
26	194	202	0.68	193	269	1.00	254	262	1.00	212	380	1.00
27	198	210	0.99	209	269	1.00	238	274	1.00	376	380	1.00
28	194	210	1.00	241	297	1.00	254	282	1.00	380	424	1.00

Table 5.3. Genotypes of newborn individuals of *S. typhle* species at the four studied microsatellite loci of the present study.
Πίνακας 5.3. Γενότυποι των απογόνων του είδους *S. typhle* όπως προέκυψαν από γενοτύπιση στους τέσσερις υπό μελέτη μικροδορυφορικούς τόπους

	S.abas3		S.abas4		S.abas7		S.abas9	
samples	**Allele1**	**Allele2**	**Allele1**	**Allele2**	**Allele1**	**Allele2**	**Allele1**	**Allele2**
S.typ1	178	206			246	278	216	256
S.typ3	214	218	237	253	222	250	220	228
S.typ7	202	214	237	237	222	250	228	268
S.typ9	178	206	233	249	250	278	220	256
S.typ14	178	206	193	249	250	278	220	228
S.typ15	178	206	237	253	222	250	220	256
S.typ16	206	214	237	237	222	234	216	256
S.typ17	178	202	249	249	246	278	228	268
S.typ18	214	218	237	253	222	238	220	228
S.typ19	214	218	193	249	238	278	220	228
S.typ22			193	237	234	278	220	256
S.typ23			237	237			216	228
S.typ27	206	214	237	237	234	278	228	268
S.typ28			237	253	246	278	228	268
S.typ51	214	218	237	253	222	238	220	256
S.typ52	178	206	233	237	250	278	256	272
S.typ59	214	218	233	237	222	250	220	256
S.typ60	206	214	249	253	238	278	228	272
S.typ61	206	214	209	249	238	278	228	272
S.typ63	178	202	237	237	234	278	228	268
S.typ64	210	214	233	237	238	278	200	228
S.typ67	210	214	209	237	222	238	228	272
S.typ68	178	202	249	249	246	278	216	256
S.typ70	214	218	193	237	222	238	200	256
S.typ71	202	214	237	237	222	234	216	228
S.typ73	178	206	237	253	238	278	200	256
S.typ75	210	214	209	249	250	278	256	272
S.typ80	214	218	237	253	222	238	200	228
S.typ81	178	218	249	253	250	278	220	256
S.typ123	206	238	221	225	198	234	212	224
S.typ124	218	238	205	229	210	254		
S.typ125	222	238	197	225	210	254	192	212
S.typ126	222	238	205	229	210	238	212	240
S.typ127	206	238	221	225	198	234	200	212
S.typ128	222	222	229	241	210	238		
S.typ129	222	222	205	229	234	238	192	256
S.typ130	234	238	225	241	234	238	240	256
S.typ131	222	238	205	229	234	254	240	256
S.typ132	206	238	221	229	198	234	200	212

S.typ133	222	222	229	237	234	254	212	256
S.typ134	222	222	225	241	210	238	212	240
S.typ135	222	230	225	229	210	266	200	212
S.typ136	222	222	229	241	234	254	192	256
S.typ137	222	222	229	241	210	254	240	256
S.typ138	218	238	229	237	234	254	212	256
S.typ139	230	238	221	229	234	266	200	256
S.typ140	230	238	221	225	198	210	212	224
S.typ141	238	238	205	229	234	254	208	212
S.typ89	226	262	233	245	230	258	200	200
S.typ90	234	262	233	245	226	258	204	224
S.typ91	226	294	233	245			204	224
S.typ93	234	294	233	245	226	258	200	204
S.typ94	226	262	221	233	226	258	200	224
S.typ95	226	294	221	233	230	250	200	200
S.typ96	234	262	221	345			204	224
S.typ97	226	294	221	345	226	250	200	200
S.typ99	226	262	221	233	230	258	200	224
S.typ100	234	294	221	233	226	250	204	224
S.typ101	226	294	221	345	230	250	200	204
S.typ102	226	294	221	233	226	250	200	204
S.typ103	186	294	221	345	230	250	204	224
S.typ104	226	262	245	345	230	258	200	204
S.typ105	226	294	245	345	226	250	200	200
S.typ106	234	294	221	345	230	250	200	224
S.typ107	234	262	221	233	226	258	204	224
S.typ108	226	262	245	345	230	258	200	200
S.typ109	226	294	233	245	226	250	200	224
S.typ110	234	294	233	245	226	258	200	224
S.typ111	234	262	221	233	230	250	200	224
S.typ112	226	262	221	345	230	258	200	224
S.typ113	234	262	233	273	226	258	204	224
S.typ114	226	262	245	345	230	250	200	200
S.typ115	234	262	221	345	230	258	204	224
S.typ116	234	294	221	233	226	250	200	204
S.typ117	226	294	221	233	226	250	200	204
S.typ118			221	233	230	258	200	204
S.typ119	226	294	221	345	230	250	200	204
S.typ120	234	294	221	233	226	250	200	204

Table 5.4. Number and genotypes of the possible mothers of *S. typhle* species as estimated from the COLONY software

Πίνακας 5.4. Αριθμός και γενότυπος των πιθανών μητέρων του είδους *S. typhle* όπως προέκυψαν από την επεξεργασία με το πρόγραμμα COLONY

	S.abas 3			S.abas4			S.abas7			S.abas9		
Moder ID	Alleles		Probability	Alleles		Probability	Alleles		Probability	Alleles		Probability
1	202	206	1.000	237	249	0.840	234	246	1.000	216	268	1.000
2	206	218	1.000	193	253	1.000	238	250	1.000	200	220	1.000
3	206	210	1.000	209	233	0.999	238	250	1.000	200	272	0.973
4	206	230	1.000	221	229	0.531	198	266	0.998	200	224	1.000
				221	225	0.304						
5	218	222	0.694	205	237	0.863	254	254	0.201	212	256	0.179
	218	218	0.047				250	254	0.135	256	256	0.172
							254	278	0.097	200	256	0.119
							234	254	0.093	200	212	0.062
6	222	238	0.507	205	241	1.000	238	254	1.000	192	240	1.000
6	222	234	0.345									
7	226	234	1.000	221	245	1.000	226	230	1.000	200	224	0.998